CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (r. 82/08)

Canada

254

0-315-12565-9

National Library of Canada | Bibliothèque nationale du Canada

Canadian Theses Division | Division des thèses canadiennes

Ottawa, Canada K1A 0N4

60476

PERMISSION TO MICROFILM — AUTORISATION DE MICROFILMER

• Please print or type — Écrire en lettres moulées ou dactylographier

Full Name of Author — Nom complet de l'auteur

RAJGOPAL RAGHUPATHY

Date of Birth — Date de naissance

JULY 17, 1957

Country of Birth — Lieu de naissance

INDIA

Permanent Address — Résidence fixe

A/2, EMERALD SOCIETY,
KALINA, BOMBAY 400098
INDIA.

Title of Thesis — Titre de la thèse

EXPRESSION AND RELEVANCE OF PATERNAL MHC ANTIGENS ON THE MURINE PLACENTA.

University — Université

U. of ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

Ph. D.

Year this degree conferred — Année d'obtention de ce grade

1982

Name of Supervisor — Nom du directeur de thèse

Dr. T. G. WEGMANN

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

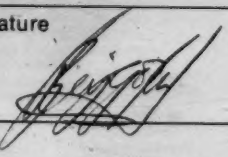
L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

Date

Oct. 14, 1982

Signature



NL-91 (4/77)

THE UNIVERSITY OF ALBERTA

EXPRESSION AND RELEVANCE OF PATERNAL MHC ANTIGENS ON THE MURINE PLACENTA

by

©RAJGOPAL RAGHUPATHY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

IMMUNOLOGY

(MEDICAL SCIENCES)

EDMONTON, ALBERTA

FALL, 1982

Microfilming

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Rajgopal Raghupathy

TITLE OF THESIS Expression and Relevance of Paternal MHC Antigens on the Murine Placenta

DEGREE FOR WHICH THESIS WAS PRESENTED Doctor of Philosophy

YEAR THIS DEGREE GRANTED Fall, 1982

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) .....

PERMANENT ADDRESS:

A/2, Emerald Society,

Kalina, Santacruz, Bombay

INDIA 400 098

DATED October 12 19 82

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Expression and Relevance of Paternal MHC Antigens on the Murine Placenta submitted by Rajgopal Raghupathy in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Immunology.

Supervisor

External Examiner

Date October 12, 1982

ABSTRACT

Mammalian pregnancy appears to contradict the laws of transplantation, because the allogeneic fetus, which may be considered to be an allograft, is maintained successfully in a potentially hostile environment during gestation. This thesis elaborates studies on certain aspects of placental immunobiology, performed to better understand the mechanisms responsible for the almost invariable success of the fetal allograft.

Since most of the fetal–maternal transactions occur at the fetal–maternal interface, i.e. in the placenta, we investigated the expression of paternally–derived major histocompatibility complex (MHC) antigens in the allogeneic placenta. Using an *in vivo* approach, we demonstrated the presence of paternally–inherited H–2K and H–2D (Class I) antigens on the placenta. These antigens are directly exposed to maternal circulation and fetectomy studies showed that these antigens originate in the placenta, at least after day 11 of gestation. Autoradiography studies showed that paternal Class I antigens are expressed by spongiotrophoblast cells in the spongy zone of the placenta, in contact with maternal decidual cells and by cells in the endodermal sinuses. The placenta appears to be lacking in paternal I–A (Class II) antigens. It is our hypothesis that the lack of cell–mediated anti–fetal immunity during pregnancy may be explained by the configuration of paternal alloantigens on the placenta – the expression of paternal Class I antigens and the absence of Class II antigens.

Several studies have documented that humoral anti–fetal MHC antibodies are generated during pregnancy and the fetus survives despite these antibodies. We have demonstrated that the murine placenta serves as an immunoabsorbent sponge by absorbing anti–fetal H–2 antibodies from maternal blood, thereby preventing these antibodies from reaching the fetus. The fact that the presence of anti–fetal H–2 antibodies does not compromise pregnancy may be attributed to the immunoabsorbent capability of the placenta. Turnover kinetic studies confirmed that the placenta is capable of re–expressing it's paternal H–2 antigens after binding anti–H–2 antibodies *in vivo*. We followed the fate of the absorbed anti–H–2 antibodies and found that these antibodies are degraded by the placenta and are then released into the circulation.

All these findings together substantiate the idea that the placenta plays a crucial role in the immunoprotection of the fetus.

PREFACE

Parts of the work described in this thesis have been published in the *Journal of Immunology* and the *Journal of Reproductive Immunology*.

ACKNOWLEDGEMENT

I shall remain indebted to many of my colleagues in the Department of Immunology. I would like to particularly thank:

Dr. Thomas Wegmann, my advisor, for his inspiring guidance and support;

Dr. Bhagirath Singh, for his invaluable suggestions and assistance;

Dr. Erwin Diener, Dr. John Barrington Leigh, and Dr. Timothy Mosmann for their assistance at different stages of my research program;

Dr. Eric Nisbet-Brown, Ms. Carla D. Cumming and Ms. Rose D. Hunzikerfor bailing me out more than a few times;

Dr. Phillip Gambel, who has been invaluable in too many ways;

and

my parents, for everything.

I gratefully acknowledge the receipt of a studentship and research allowance from the Alberta Heritage Foundation for Medical Research during 1980-82.

Table of Contents

Chapter		Page
I.	INTRODUCTION	1
	A. Transplantation Biology	1
	B. Embryogenesis and Placentation	3
	C. The Uterus as an Immunologically Privileged Site	8
	D. Antigenicity of the Embryo and Placenta	10
	Histocompatibility Antigens	11
	Tissue-Specific Antigens	18
	Oncofetal Antigens	19
	E. Maternal Anti-Fetal Reactivity	21
	Morphologic Alterations in Lymphoid Organs during Pregnancy	22
	Maternal Anti-Fetal Cell-Mediated Immunity	23
	Maternal Anti-Fetal Humoral Immunity	24
	F. Immunoregulation during Pregnancy	26
	G. Fetal-Maternal Cell Trafficking	29
	H. The Placenta as a Cell Filter	30
	I. The Placenta as an Antibody Filter	31
II.	MATERIALS AND METHODS	33
III.	RESULTS	41
	A. Testing of Anti-H-2 Antibodies	41
	B. Testing of Radiolabeled Antibodies	41
	C. Evidence for the Placental Immunoabsorbent Model	41
	D. Quantitation of Paternal H-2K Antigens on the Placenta	47
	E. Quantitation of Paternal H-2D Antigens on the Placenta	57
	F. Binding of $F(ab)_2$ Anti-H-2K by the Target Placenta	57
	G. Effect of Fetectomy on Placental Immunoabsorption of Anti-Paternal H-2K Antibody	60
	H. Autoradiographic Localization of Paternally-Derived H-2 Antigens in the Placenta	64
	I. Antibody Turnover in the Placenta	71
	J. Fate of Anti-H-2 Antibody in Target and Control Placentas	72

Analyses of Serum for IgG Degradation Products 78
Anti-H-2 Antibody Degradation in the Liver 79
K. Examination of Paternal I-A Antigens on the Allogeneic Placenta 79
IV. DISCUSSION 82
V. CONCLUSIONS 99
VI. REFERENCES 101

LIST OF TABLES

Table 1 – H-2 haplotypes of the mouse strains and regions of the H-2 complex recognized by the antibodies used in these studies 34

Table 2 – Weights of placentas obtained from fetectomized and non-fetectomized uterine horns 63

Table 3 – Effect of fetectomy on placental immunoabsorption of anti-paternal antibodies 65

Table 4 – Lack of expression of paternal I-A antigens by the allogeneic placenta 81

LIST OF FIGURES

Figure 1 – Diagrammatic representation of the conceptus 6

Figure 2 – Anatomical structure of the hemochorial placenta 7

Figure 3 – Competitive inhibition assay to assess antibody activity 42

Figure 4 – Quantitative absorption of radiolabeled anti-H-2K antibody 43

Figure 5 – Evidence for the placental immunoabsorbent model 45

Figure 6 – Testing of immunoabsorbent antibody kinetics using F(ab')$_2$ anti-H-2 antibodies 46

Figure 7 A – Time course of the binding of anti-H-2K antibodies to placentas on day 10 of gestation 48

Figure 7 B – Time course of the binding of anti-H-2K antibodies to placentas on day 13 of gestation 49

Figure 7 C – Time course of the binding of anti-H-2K antibodies to placentas on day 17 of gestation 50

Figure 8 A – Binding of anti-paternal H-2 antibodies to placentas on day 10 52

Figure 8 B – Binding of anti-paternal H-2 antibodies to placentas on day 13 53

Figure 8 C – Binding of anti-paternal H-2 antibodies to placentas on day 17 54

Figure 9 – Comparison of the specific binding of antibody to target and control placentas at different stages of gestation 55

Figure 10 – Determination of the placental immunoabsorptive capacity for anti-paternal H-2K antibodies 56

Figure 11 – The estimated density of paternal H-2K antigens on the placenta at different stages of gestation 58

Figure 12 – Binding of radiolabeled anti-H-2D antibody to target and control placentas 59

Figure 13A – Binding of F(ab')$_2$ anti-H-2K to target and control placentas 61

Figure 13B – Determination of placental immunoabsorptive capacity for F(ab')$_2$

anti-paternal H-2K antibodies 62

Figure 14 - Radiolabeled parietal endoderm cells within the endodermal sinuses of the placenta 66

Figure 15 - Radiolabeled degenerating cells in the endodermal sinuses 67

Figure 16 - Autoradiogram revealing densely labeled cells of monocytic or macrophage-like appearance in the placenta 68

Figure 17 - Autoradiogram revealing labeled spongiotrophoblast cells in the spongy zone of the placenta 70

Figure 18 - Autoradiogram revealing labeled spongiotrophoblast cells 71

Figure 19 A - Determination of the dose of unlabeled antibody required to inhibit the *in vivo* binding of radiolabeled anti-H-2K to the placenta 73

Figure 19 B - Determination of the time required for the placenta to regain its immunoabsorptive capacity for anti-H-2K antibody. 74

Figure 20 - Fate of anti-H-2K*k* antibodies 1 hour after injection 75

Figure 21 - Fate of anti-H-2K*k* antibodies 4 hours after injection 76

Figure 22 - Fate of anti-H-2K*k* antibodies 6 hours after injection 77

Figure 23 - Fate of anti-H-2K*k* antibodies in the liver 80

LIST OF ABBREVIATIONS

AFP	alpha fetoprotein
cpm	counts per minute
CTL	cytotoxic T lymphocyte(s)
DLN	draining lymph node(s)
EPC	ectoplacental cone
gm	gram(s)
GVHR	graft versus host reaction
H	histocompatibility
hCG	human chorionic gonadotropin
ICM	inner cell mass
i.v.	intravenous
MEM	minimum essential medium
mg	milligram(s)
MHC	major histocompatibility complex
MiH	minor histocompatibility
ml	milliliter
MLR	mixed lymphocyte reaction
PBS	phosphate buffered saline
PHA	phytohemagglutinin
PMSF	phenylmethyl sulphonyl fluoride
RBC	red blood cell(s)
ug	microgram(s)
ul	microliter(s)

I. INTRODUCTION

In outbred mammalian populations, a significant immunologic contradiction exists during pregnancy. The uterus of the mother harbors a genetically dissimilar conceptus, which is essentially an allograft. Yet the fetus almost invariably survives, unharmed by the maternal immune system. The mechanisms underlying the enigmatic survival of the fetal allograft have been one of the preoccupations of reproductive immunologists for about three decades.

The objective of this chapter is to review the literature pertaining to maternal–fetal immunology and especially to my research. This work essentially deals with the role of the placenta in maternal–fetal interactions. Therefore this and other related aspects will form the major focus of this chapter.

A. Transplantation Biology

At the turn of this century, biologists interested in cancer research, were seeking to prolong the life of experimental tumors by transplanting neoplastic tissue from one animal to another. Only a few of the tumors survived for prolonged periods of time while and most were rejected; the conditions under which this occured were not known. The advent of inbred stocks of mice, introduced by Jensen (1903) helped in understanding this phenomenon. It was observed that transplanted tumors grew well in inbred strains of mice, only if the tumor transplant was made between members of the same strain. If tumors of other mice were transplanted to mice of the inbred strain, they were rapidly rejected (Loeb, 1908; Tyzzer, 1909). Thus the importance of "strains" in susceptibility to tumor transplants was realized and a genetic control of tissue transplantability was suggested. Haldane (1933) was the first to suggest that acceptance or rejection of these tumor grafts might have an immunological basis. He also postulated that the immunity was directed at alloantigens on the graft rather than at tumor–specific antigens. He predicted that similar antigenic differences exist in other tissues and that the alloantigens induce an immune response in hosts lacking them. Gorer (1938) provided evidence for the immunological nature of resistance to tumor transplants by showing that rejection of a tumor is accompanied by the production of alloantibodies and also demonstrated that the

genes for susceptibility to tumor transplants were identical with genes coding for alloantigens. Gorer (1938) put forward an immunological theory of transplantation by stating that "iso-antigenic factors present in the grafted tissue and absent in the host, are capable of eliciting a response which results in the destruction of the graft".

The second major series of elegant experiments which linked the rejection of transplants to immunoreactivity came from the work of Medawar and his colleagues. The development of the rejection reaction and the resultant state of immunologically specific memory, was shown to be systemic in nature (Medawar, 1944). This was followed by intensive search for the immunologic factors involved in transplant rejection. Even though humoral antibodies were frequently found in recipients that rejected normal tissue allografts (Amos *et al*, 1954), it was gradually realized that the humoral antibody response was not the full explanation of graft rejection. In a classical adoptive transfer experiment, Mitchison (1953) demonstrated that rejection of tissue grafts was mediated by and could be passively transferred with, lymphocytes. Antibodies and cell-mediated mechanisms both play a role in the rejection of allografts and the relative role of each mechanism depends on the grafted tissue and the species, strain and state of immunity of the host.

The sequence of events leading to graft rejection is not absolutely clear. Host lymphocytes may enter the graft, become triggered by donor antigens and attract other cells to the graft site. Alternatively, donor lymphocytes from the graft may enter the host circulation, get trapped in the draining lymph node and may then stimulate host immune responses in the node. Sensitized host lymphocytes may then enter the graft site; lymphocytes begin to attack graft cells, and gradually the connections between donor and host vessels degenerate. The graft becomes indurated and separates from the graft bed.

In summary, syngeneic grafts are accepted and allogeneic grafts are rejected. In the context of transplantation immunobiology, the immunological problem of pregnancy was elaborated and addressed for the first time by Medawar in 1953. He asked : "How does the pregnant mother contrive to nourish within itself, for many weeks or months, a fetus that is an antigenically foreign body?" This question has continued to intrigue many workers in this area and has yet to be unequivocally resolved. In outbred populations, the fetus is almost invariably semiallogeneic to the mother. Allogeneic and semiallogeneic

grafts differing at the MHC haplotype, are rejected, yet the allogeneic fetus appears to defy the laws of transplantation. The major hypotheses that have been put forth to explain how the fetal allograft may evade maternal rejection are :

(1) The pregnant uterus is an immunologically privileged site. Grafts to this site are shielded from the maternal immune system.

(2) The embryo is "antigenically immature", that is, it does not express transplantation antigens at the cell surface.

(3) The maternal response to immunogens on the conceptus is weakened or suppressed either specifically or non-specifically.

(4) The developing embryo is shielded from maternal immune responses by extraembryonic layers, notably the trophoblast. (Medawar, 1953; Billingham, 1964; Edidin, 1977).

Innumerable experiments have been performed during the last two decades in attempts to substantiate or disprove each of these models. Before entering into a discussion of these models and their relevance, we may now briefly outline the development of the embryo and the placenta.

B. Embryogenesis and Placentation

The embryo begins as an egg which becomes fertilized in the upper part of the fallopian tube by a single sperm, resulting in the formation of a zygote. Fertilization is followed by a series of mitotic cleavages, the first cleavage taking place approximately twenty four hours after fertilization. Cleavage of zygotes results in the formation of progressively smaller cells, called blastomeres. By the third day after fertilization the embryo develops into a clustered group of eight or more cells, the morula, held together and protected by the zona pellucida, which is an acellular membranous sheath. Early on the fourth day of development, the morula arrives in the uterus via the oviduct where the zona pellucida disappears within twelve hours. The morula becomes converted into a unilaminar blastocyst by a process called cavitation. Cavitation is the development of a split between the outer and inner layer of cells which spreads in all directions until it has detached the surface cells from the inner cells everywhere but at the boundaries of the inner cell mass (ICM). The blastocyst therefore consists of an outer rim of extra-embryonic

trophectoderm cells, containing a cluster of undifferentiated embryonic protodermal cells of the ICM protruding into the blastocoele cavity. The ICM eventually develops into the fetus and the trophectoderm develops into extraembryonic tissues.

The blastocyst establishes contact with uterine mucosa by the fifth day of murine gestation by a process called implantation. Trophoblast cells insert narrow processes which squeeze through the uterine epithelial layer and penetrate the basement membranes of the uterine epithelium, finally coming to rest with decidual cells. Decidual cells are supposed to be uterine stromal fibroblasts which are transformed as a result of blastocyst attachment. Decidual cells are arranged as a solid mass of epitheloid cells forming an implantation chamber at the site of implantation. The mural trophoblast cells surrounding the blastocoele enlarge and are transformed into primary giant cells which function as an anchoring structure during the early stages of implantation (Billington, 1975). The polar trophoblast cells overlying the ICM remain unchanged and diploid, and give rise to a conical mass of cells, the ectoplacental cone (EPC). The EPC is composed of a group of highly invasive proliferative core of diploid cells and an outer layer of polyploid, secondary giant cells (Rossant and Papaionnou, 1977). The secondary giant cells migrate outwards to lie at the margins of the developing placenta and downwards to surround the early embryo. The proliferative center of the EPC differentiates to give rise to the the main trophoblastic components of the definitive murine placenta (Billington, 1975). The word placenta (meaning "flat cake") is applicable to any combination of embryonic and maternal tissues, which serves as the medium of physiological interchanges between the mother and fetus (Hendricks and Houston, 1970).

The functional attachment of the placenta to the uterus is in the shape of a round or oval disc in rodents and in primates, and the placenta is therefore described as being discoid. The placenta appears to have three main functions in maintaining pregnancy; these include transport of inorganic substances, gases, water, organic nutrients and metabolic wastes, the storage of lipids, vitamins, hormones and metabolism of glycogen, amino acids and lipids required for the embryo, and the biosynthesis of steroids and gonadotropins.

The placenta is made up of three trophoblastic cell types. These are:
(i) the spongiotrophoblast, which abuts uterine decidual tissues intimately and is about

three or four cell layers in thickness,

(ii) trophoblastic giant cells, which lie at the maternal boundary of the spongiotrophoblast and

(iii) the labyrinthine trophoblast which is the region where the main maternal blood flow occurs (Figure 1).

The labyrinthine trophoblast is trilaminar; the outer layer is cellular and is bathed by maternal blood in the placental sinuses and the two inner layers are apparently syncytial (Enders, 1965). The labyrinthine trophoblast region is vascularized by fetal blood vessels while the spongy region is not. Maternal capillaries in both regions end in blood sinuses. These sinuses are lined by the three layers of the murine trophoblast which separate the two blood streams. The outermost of the trophoblast layers form the walls of the maternal blood sinuses while the innermost cells rest upon a basement membrane which intervenes between the chorionic epithelium and the fetal capillaries (Amoroso, 1964) (Figure 2). Thus in all mammalian species the trophoblast is the only fetal element of the placenta that remains in direct and continuous contact with the maternal tissues, either maternal decidual cells or maternal blood. The placenta in rodents and primates is classified as being hemochorial indicating that there is a very close association of maternal and fetal tissues and that maternal blood bathes the trophoblast directly (Figure 2).

The organization of the human placenta is somewhat different. After implantation, the trophectoderm of the human blastocyst gives rise to two basic forms of trophoblast – an inner cellular cytotrophoblast and an outer syncytiotrophoblast which is formed by the fusion of cell membranes to build a syncytium with the resulting nuclei scattered through a continuous cytoplasm. The cytotrophoblast pushes through the syncytiotrophoblast and forms villi which develop intimately with an outer syncytial covering and an underlying cytotrophoblast layer. Thus, in the human placenta, the villous syncytium is bathed in maternal blood and the cytotrophoblast is in contact with uterine decidual tissue. The chorionic membrane, composed mainly of a layer of cytotrophoblast cells, surrounds the remainder of the conceptus after the main placental region is established (Billington, 1975).

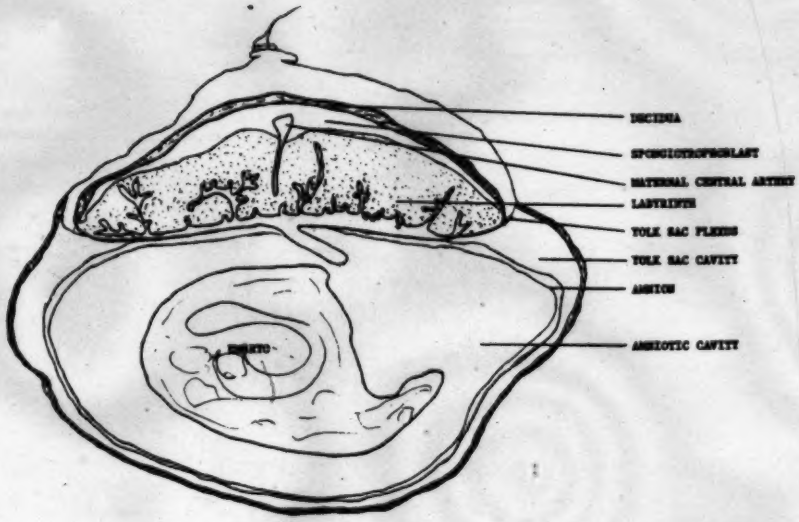


Figure 1 – Diagrammatic representation of the conceptus depicting the decidua, spongiotrophoblast and labyrinthine trophoblast regions of the murine placenta in relation to the embryo. (Adapted from Rugh, 1968)

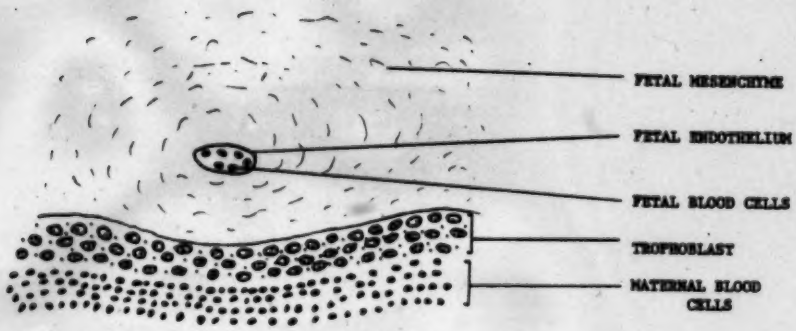


Figure 2 – Anatomical structure of the hemochorial placenta. (Adapted from Gill and Repetti, 1979).

In the meanwhile, during placentation and later, each of the three germ layers in the murine and human embryo develop into smaller groups of cells which are destined to form a certain organ or organ system – a process called organogenesis. During organogenesis, the shape and size of the embryo changes. There is an increase in cell number and cell size which enables the animals to gradually achieve the size of its parents. The murine gestation period ranges between 18 and 21 days.

We may now consider the different mechanisms which have been suggested as being responsible for the success of the fetal allograft.

C. The Uterus as an Immunologically Privileged Site

Medawar (1953) suggested that the pregnant uterus may serve as an immunologically privileged site, protecting the fetus by bringing about a delay in either the recognition of the fetus as foreign, or by preventing the entry of maternal humoral and cellular effectors into the fetus.

Tissues transplanted into a number of specialized sites like the brain, the anterior chamber of the eye and the hamster's cheek pouch, survive for extended periods of time. This immunological privilege is due to a lack of vascular supply or drainage which prevents the immediate recognition of foreign antigens in the grafted tissue by the immune system of the host (Billingham and Silvers, 1964). The privileged status of the hamster's cheek pouch is similarly attributed to a lack of lymphatics connecting this site to the regional lymph nodes, with a consequent lack of transport of antigenic material to the lymph nodes and likewise of sensitized T cells to the cheek pouch. It has been suggested that the fetal allograft may enjoy a similar privilege in the uterus, wherein the afferent and/or the efferent limb of the immune response arc may be blocked (Barker and Billingham, 1971).

Physiologic or immunologic modifications occuring in the uterus during pregnancy may result in some sort of protection being afforded to the developing fetus (Beer and Billingham, 1976). Initially, Beer and Billingham (1971) had established that histoincompatible skin cylinders were able to heal in effectively onto the uterine mucosa of non-pregnant females, but were rejected as quickly as if they had been transplanted to a more conventional site; therefore the uterus appears to be immunologically

competent. However, skin allografts enjoyed extended survival within a *pregnant* uterus, as opposed to orthotopic skin grafts. Similarly, lymphocytes injected into the uterus of non-pregnant mice, can elicit systemic responses leading to an accelerated rejection of skin grafts; however, female mice are not sensitized by intra-uterine injections of lymphocytes *during* pregnancy, suggesting that physiologic or immunologic modifications occuring in the uterus as a result of pregnancy, may in some way protect the developing fetus (Beer and Billingham, 1971). Further studies by Beer and Billingham (1974) provided evidence for the thesis that increased hospitality afforded to skin allografts healed in on decidual tissue in non-sensitized hosts was due in part to an afferent, and not efferent, blockade of the draining uterine lymphatic vessels. This conclusion was based on the finding that the skin allografts in the uterus were promptly rejected when the animals were adoptively given specifically immune lymphoid cells. These findings cumulatively suggest a possible crippling of the effector arm of the immune response arc. Furthermore, decidual cells may cause this afferent blockade but these cells do not seem capable of blocking the efferent limb of the uterine immune reflex arc (Beer and Billingham, 1974). Allogeneic embryos appear to be protected when transplanted into the uterus, but are rejected when transplanted into ectopic sites in experimentally immunized female mice (Kirby *et al*, 1966). Blastocysts from C57Bl x C57Bl matings were transplanted to the kidneys and to the pseudopregnant uterus of specifically hyperimmunized C3H mice. All the transplants to the kidney failed to grow, although control grafts of isologous blastocysts to the uterine site implanted and developed to term. Kirby *et al* (1966) interpreted these results as evidence for the role played by decidual cells in protecting the blastocysts from maternal effector mechanisms.

The maternal immune system is often sensitized to fetal antigens during pregnancy, but the induction of such immune effectors during pregnancy, and experimental sensitization of the maternal immune system does not seem to compromise pregnancy (Beer and Billingham, 1976). The argument for an involvement of decidual cells in protecting the conceptus by rendering the uterus refractory to immunologic attack is strong, but this question has yet to be resolved conclusively. Decidual tissue is vascularized and is permeated by lymphatic vessels, hence the uterus is unlike the anterior chamber of the brain or the hamster cheek pouch, which are classical examples

of immunologically privileged sites. Thus it would be fair to conclude that while the maternal immune system can be sensitized via the intrauterine route, the induction of a graft rejection reaction is probably modified by anatomic, physiologic or immunologic characterisitics (Beer and Billingham, 1976).

D. Antigenicity of the Embryo and Placenta

Little (1924) suggested that the embryo does not develop it's individual antigenic characteristics until relatively late in gestation, thereby preventing the stimulation of an effective maternal immune reaction, or the destruction of embryonic or fetal tissues by any pre-existing immunity. This hypothesis was also extended by Medawar (1953) who suggested that rats and mice are in an embryological sense relatively immature at birth and that this embryonic immaturity may be reflected as hypoantigenicity of the embryo. Ingenious as this model is, it has been challenged by investigations that have clearly demonstrated "foreign" antigens on both embryonic and extra-embryonic tissues.

A proportion of primiparous and a higher proportion of multiparous females of a variety of species manifest immune responses directed at fetal and placental antigens indicating the presence of antigens foreign to the mother, in the conceptus. This however does not provide any insight into the actual tissues - fetal or placental - which evoke such responses in the mother. Direct investigations into the antigenicity of embryonic tissues have resulted in a firm consensus that the embryo is antigenically mature in some respects, and does express antigens that are foreign to the mother.

Embryonic antigens that may be involved in maternal-fetal interactions can be grouped into three categories:

(i) paternal histocompatibility antigens,

(ii) tissue-specific antigens, and

(iii) oncofetal antigens.

Histocompatibility Antigens

The Major Histocompatibility Complex (MHC) is a chromosomal segment that codes for polymorphic products that are responsible for the rapid rejection of foreign tissue grafts. This complex is designated as the H-2 complex in the mouse and as the HLA system in man. The H-2 complex is divided into two classes of loci, Class I comprising H-2K, H-2D and H-2L encoding loci while Class II consists of I-A and I-E encoding loci (Klein et al, 1981). Class I loci differ from Class II loci in that the former in general serve as targets for cytolytic T cells and the latter generally guide regulatory (helper and suppressor) T cells (Klein et al, 1981). Two other loci have been supposed to be present in the H-2 complex; Klein (1981) suggests that I-J loci may be included after confirmation of its relation to the H-2 complex, and the I-E loci may be included after confirmation of its existence. Products of the I-A and I-E loci (Class II antigens) are involved in a variety of immune recognition and T-B lymphocyte co-operative phenomena (Moller, 1976) in graft-versus-host reactions (Klein and Park, 1973) and in graft rejection (Klein *et al*, 1977). The human HLA-A, -B, -C and -DR genes correspond to murine H-2K, H-2D, H-2L and Ia genes repectively (Strominger, 1980).

In addition to the MHC antigens, there is another group of antigens, the Minor Histocompatibility (MiH) antigens, which differ from the MHC antigens in that allografts transplanted to hosts differing in MiH antigens are rejected after substantially longer periods of time.

Several investigators have examined the expression of paternal major and minor histocompatibility (H) antigens by the murine embryo and placenta at different stages of gestation primarily because of the crucial involvement of these antigens in allograft rejection phenomena. The oocyte was initially reported to be devoid of H-2 antigens (Billington *et al*, 1977); however, Heyner and Hunziker (1979) reported the detection of H-2 antigens on oocytes by the indirect immunofluorescence technique. In contrast to the finding of Class I antigens, Heyner and Hunziker (1982) were unable to detect Class II MHC antigens on the oocyte using their immunofluorescence assay.

After fertilization, significant changes occur on the plasma membrane of oocytes (Johnson and Edidin, 1978); activation of the embryonic genome occurs very early in murine embryonic development, probably immediately after fertilization and certainly

after two divisions (Solter and Knowles, 1979).

In contrast to the oocyte, the surface of the two-cell stage embryo was found to lack MHC antigens by Heyner *et al* (1980). They suggest that this lack of antigenicity might help the embryo elude recognition by the maternal immune system during this especially vulnerable stage of development. The eight-cell embryo is also devoid of, or is deficient in, H-2 antigens as shown by the cytotoxicity studies of Heyner *et al* (1969).

Paternal H-2 antigens appear for the first time in the inner cell mass (ICM) of the peri-implantation murine blastocysts as demonstrated by immunofluorescence studies (Heyner 1973) and by transplantation studies (Patthey and Edidin, 1973). Webb *et al* (1977) using sensitive immunoprecipitation techniques, observed that only the ICM of the late blastocyst is capable of *de novo* synthesis of H-2 antigens. Most investigators seem to agree that the trophectoderm is devoid of paternal MHC antigens and that only the inner cell mass of the late blastocyst expresses H-2 antigens. However, Searle *et al* (1976) reported a *transient* expression of H-2 antigens by the trophectoderm of pre-implantation stage blastocysts. These workers used a sensitive immunoperoxidase assay and found that the expression of paternal H-2 antigens by the trophectoderm was relatively weak; they attributed this positive reactivity to a greater resolution and sensitivity of their assay. Hakannson *et al* (1975) used an antiglobin assay to study the expression of H-2 antigens on blastocysts undergoing a delay of implantation and reported a variable expression of MHC antigens on the trophectoderm. Release from delay of implantation resulted in abolishment of H-2 antigen expression on the trophectoderm. Similarly, Searle *et al* (1976) reported the lack of these antigens on the post-implantation ectoplacental cone (EPC) trophoblast. The expression of MHC antigens by the blastocyst trophectoderm is therefore abolished after implantation.

At implantation, the zona pellucida is shed and the blastocyst attaches to the uterine wall which degenerates and then permits the trophectoderm to come into close apposition with uterine stroma and maternal blood. Lack of strong transplantation antigens on the blastocyst at this stage when maternal and fetal tissues are actually juxtaposed for the first time in embryonic life, will conceivably reduce the possibility of maternal immune recognition and immunologic attack.

Minor Histocompatibility (MiH) antigens have been demonstrated on blastocysts by the cytotoxicity assay (Heyner *et al*, 1969), by indirect immunofluorescence (Palm *et al*, 1969) and by transplantation experiments (Searle *et.al*, 1974). Heyner *et al* (1980) recently reported the results of immunofluorescence studies performed using extensively absorbed antisera; MiH antigens were first detectable on the eight-cell stage and subsequently on morulae, blastocysts and blastocyst trophectoderm. One other histocompatibility-associated antigen that has been detected on the mouse embryo, is the H-Y antigen which is seen on 51% of embryos starting from the 6 to 8-cell stage (Krco and Goldberg, 1977). MiH antigens are important in this context because these antigens can cumulatively present sufficient alloantigenicity so as to invoke strong host-versus-graft rejection reactions (Graff, 1978); the survival of embryos despite the presence of "foreign" MiH antigens is surprising, and may be attributed to a low level expression of these antigens (Heyner, 1982).

The presence of H-2I products on the developing blastocyst has also been investigated in a few studies. Jenkinson and Searle (1979) examined the expression of allo-Ia antigens on pre-implantation and early post-implantation embryos and on the trophoblast of the definitive placenta, usiing an immunoperoxidase assay and were unable to detect any paternal Ia antigens on all the stages of gestation studied. The absence of paternal Ia antigens may be beneficial to embryonic survival, as I region differences between the embryo and the mother may stimulate strong cell-mediated reactions directed at the fetus.

To summarize antigen expression during murine pre-implantation development, paternally-derived H-2 and Ia antigens are generally absent from the surface of the early embryo and the trophectoderm of the late blastocyst, except perhaps for a transient expression just prior to implantation; H-2 antigens are expressed by the ICM of the late blastocyst. Minor H antigens are present on the blastocyst apparently throughout embryonic development beginning at the morula stage.

The placenta and the outermost fetal membranes (and not the embryo itself) are juxtaposed in intimate and continuous contact with the potentially hostile maternal environment; hence, the antigenic status of the definitive placenta and of the trophoblast in particular, is of importance. The alloantigenicity of the ectoplacental cone of the

post-implantation embryo and the trophoblastic layers of the definitive placenta has been investigated by a number of workers.

The EPC trophoblast has been consistently shown to be devoid of H-2 antigens. The early mouse trophoblast does not succumb to specific pre-existing immunity in the host (Simmons and Russell, 1967). Allogeneic ectopic transfer of the EPC does not result in an inhibition of trophoblastic growth, indicating a complete absence or at least a relative intrinsic deficit of MHC antigens on the early trophoblast. Jenkinson and Billington (1974) used a cell-mediated cytotoxicity assay to examine the susceptibility of the early trophoblast to immune effector cells and they compared the susceptibility of the trophoblast with that of other embryonic tissues. Cells of the embryonic sac were killed by the effector cells in the cytotoxicity assays while trophoblastic outgrowths were unaffected. Jenkinson and Billington (1974) concluded that the 7.5 day EPC trophoblast does not express in appropriate form the same target antigens that are recognized on the cells of the embryonic sac, by cytotoxic cells. This was further supported by Pavia *et al* (1981) who attempted to immunize mice with semiallogeneic EPC trophoblast cells; they were unable to generate cytotoxic cells directed against paternal H-2 antigens. Thus, there clearly appears to be a consensus regarding the lack of paternal H-2 antigens on the EPC trophoblast. Sellens *et al* (1978) conducted mixed hemadsorption studies to examine the expression of paternal MHC and MiH antigens on explants from 7.5 day EPCs. These studies confirmed the absence of MHC antigens on the EPC trophoblast; however, MiH antigens were detectable. Thus paternal minor histocompatibility antigens appear to be expressed on the developing zygote and extra-embryonic membranes throughout gestation.

The EPC trophoblast in turn proliferates and differentiates into the definitive placental trophoblast consisting of (i) the trophoblastic giant cells in contact with maternal decidua, (ii) the spongiotrophoblast also in contact with maternal decidua and maternal blood, and (iii) the labyrinthine trophoblast bathed in maternal blood. In contrast to earlier trophoblastic stages, the definitive placenta appears to express paternally-derived MHC antigens. MHC and MiH antigens were demonstrated on 13 day placental cells by the mixed hemadsorption assay (Sellens *et al*, 1978). Monolayer cultures were prepared from 13-14 day placentas and these cultures analyzed for the presence of paternal H-2

and minor H antigens. All cells were found to express minor H antigens; giant cells (identified on the basis of morphologic criteria) were devoid of H-2 antigens. Some smaller cells, putative trophoblast cells, expressed low levels of paternal H-2 antigens. However, microscopic examination of placental cells in suspension to identify trophoblast cells is a questionable approach, hence it is not possible to draw unequivocal conclusions as to the precise cell type expressing these antigens. Sellens *et al* (1978) do argue however, that since the predominant cell type in short term cultures is trophoblastic, the paternal H-2 antigen-bearing cells in their cultures were trophoblastic. Jenkinson and Owen (1980) reported studies investigating the expression of paternally-inherited H-2 antigens by the two major trophoblastic elements of the definitive placenta - the labyrinthine and spongy trophoblasts. The spongiotrophoblast layer was trimmed off under a dissecting microscope and putative labyrinthine tissue samples were cut out from the embryonic surface. Single cell suspensions were then prepared and analyzed for the expression of paternal antigens by a mixed hemadsorption assay. These investigators reported low levels of paternal H-2 antigens on the spongiotrophoblast (which is not infiltrated by fetal blood and fetal mesenchyme). The labyrinthine trophoblast (which contains fetal blood vessels and mesenchyme) appeared to be relatively deficient in, or perhaps completely devoid of, paternal H-2 antigens. Some cells from the labyrinthine region reacted weakly with anti-H-2 antibodies but whether these cells were mesenchymal or trophoblastic in origin was not established. The drawback of all the abovementioned approaches is the possibility of contamination of one population of cells by another; the spongiotrophoblast layer is about 3 to 4 cells thick, hence experimental separation of such minute portions of tissues may never be absolute. Furthermore, Jenkinson and Owen (1980) did not establish whether these antigens are actually exposed to maternal circulation and this is an important point in the context of maternal recognition of fetal antigens. Chatterjee-Hasrouni and Lala (1979) have reported the expression of paternal H-2 antigens by trophoblast cells of the placenta by employing a quantitative autoradiographic technique to analyze the binding of anti-H-2 antisera to placental cell suspensions. These workers used histological criteria to identify trophoblast cells among a variety of other placental cells in suspension, and the precise identification of trophoblast versus fetal mesenchyme versus decidual cells in

dispersed placental cell suspensions is difficult (Beer, 1980).

Pavia *et al* (1981) examined the ability of trophoblast cells from late gestational placentas to induce cellular immune reactivity towards H-2 antigens of paternal strain origin. They found that 17 to 20 day placental trophoblast cells are capable of inducing specific immunity. However, the *in vitro* generation of cytotoxic effector cells as well as a significant proliferative response occured only if *in vivo* priming was followed by *in vitro* restimulation. In addition, these trophoblast-induced responses were lower in comparison to the responses stimulated by adult spleen cells. Pavia *et al* (1979) concluded that the level of transplantation antigens on the placental trophoblast is relatively low; Chatterjee-Hasrouni and Lala (1979) however have claimed that the density of alloantigens on the trophoblast is equivalent to that on adult thymocytes. To resolve this discrepancy Pavia *et al* (1981) suggest that these antigens may exist in a form not easily recognizable by sensitized lymphoid cells

Using an *in vivo* approach for the first time, Wegmann *et al* (1979a, b) demonstrated that the allogeneic murine placenta does express paternally derived H-2 antigens and that these antigens are accessible to maternal circulation. The presence of H-2 antigens was confirmed using monoclonal antibodies directed against paternal strain H-2K determinants. This *in vivo* procedure consisted of injecting radiolabeled anti-paternal strain H-2K antibodies into control and target pregnant mice. Wegmann *et al* (1979b) found a substantial difference in the binding of these antibodies to target (antigen-bearing) and control placentas. $F(ab')_2$ preparations of this antibody behaved in the same manner as the intact antibody, confirming the specificity of binding to the placenta. This was the first demonstration of paternally derived transplantation antigens, actually exposed to maternal circulation in the mouse placenta. They did not specify the exact cell types involved in the absorption of anti-paternal antibodies from maternal blood.

In contrast to the expression of paternal H-2 antigens, the placenta has been reported to be devoid of paternally derived Ia antigens. Jenkinson and Searle (1979) conducted immunoperoxidase studies to determine whether anti-Ia antibodies could bind to the placenta; these studies revealed that paternal Ia antigens are not present on trophoblastic tissues obtained from early post-implantation embryos and from definitive

placenta. Chatterjee-Hasrouni and Lala (1981) recently examined the presence of paternal Ia antigens on the surface of trophoblast cells by a quantitative autoradiographic technique. They were unable to detect paternal Ia antigens on trophoblast cells at gestational ages ranging between 12 and 17 days. Ia antigens first appear on embryonic cells 11 days post-conception and their expression is confined to the fetal liver until day 16 of gestation (Delovitch *et al*, 1978).

In summary, paternal Class I MHC antigens appear to be expressed by the allogeneic murine placenta, while Class II MHC antigens are not detectable on the placenta.

The situation in the human placenta is more complicated. Low frequency sonication of spleen, lung, liver and kidney from 12-22 week fetuses yields HLA antigens capable of inhibiting anti-HLA antisera (Pellegrino *et al*, 1970). These and other studies (reviewed by Loke, 1978) have demonstrated clearly that alloantigens are present in immunologically effective concentrations on human fetal tissues. A number of other studies have demonstrated paternal HLA antigens on the placenta *per se*; the as yet unresolved question is whether the *trophoblast* expresses paternal HLA antigens. Significant differences exist between murine and human placentas. The human chorionic villous trophoblast resembles the labyrinthine trophoblast of the murine placenta and the syncytiotrophoblast is bathed by maternal blood, while chorionic cytotrophoblast cells are in actual contact with decidual cells.

A number of studies have reported the absence of HLA antigens on the human trophoblast. Goodfellow *et al* (1976) examined purified preparations of placental plasma membranes and were able to demonstrate paternally-derived HLA-A and HLA-B antigens, albeit at lower levels than on splenic lymphocytes. They were unable to draw any firm conclusions as to the precise localization of these antigens. Faulk and Temple (1977) used monospecific heterologous antisera to human beta-2 microglobulin (a plasma protein invariably associated with the HLA molecule) to study the expression of beta-2 microglobulin and thereby of HLA antigens in human chorionic villi. They employed traditional immunofluorescence and immunoperoxidase methods and found that beta-2 microglobulin is not expressed by the human trophoblast. The exposure of cryostat sections of the placenta to several enzymes including neuraminidase, failed to reveal beta-2 microglobulin, suggesting that former concepts of masked MHC antigens on

trophoblasts were probably incorrect. Cells in the mesenchymal stroma of chorionic villi, including fibroblast endothelium and Hofbauer cells were positive for beta-2 microglobulin. In addition Faulk *et al* (1977) reported that rabbit and monkey anti-HLA sera showed the same distribution pattern as beta-2 microglobulin, indicating that paternal HLA antigens are perhaps not expressed by the human trophoblast.

However, not all workers agree that there is a lack of paternal HLA antigens in the human trophoblast. Loke *et al* (1971) reported the presence of HLA antigens on trophoblastic villi, detected by cytotoxicity assays performed on cultured trophoblast cells. Montgomery and Lala (1981) have recently reported the binding of anti-HLA antibodies to placental cells using a sensitive autoradiographic assay; they found weak but nevertheless positive labeling for HLA -A, -B and -C antigens. Both these aforementioned findings suffer from a drawback- that of possible contamination by non-trophoblastic placental cells in the cultures. Sunderland *et al* (1981) recently reported immunoperoxidase studies using monoclonal anti-HLA -A, -B, - C and DR antibodies to stain sections of human placenta. They found that fetal cytotrophoblast cell columns protruding from chorionic villi in the early placenta express HLA antigens. This then is the first convincing demonstration of HLA antigens on human trophoblast cells in direct contact with maternal tissue. The principal form of human trophoblast in contact with maternal decidua is the chorionic villous trophoblast and this tissue was found to lack major HLA antigens (Sunderland *et al*, 1981).

It is clear therefore that the expression of paternal HLA antigens on the human trophoblast is somewhat equivocal.

Tissue-Specific Antigens

Tissues like the testis, brain and cornea express tissue-specific antigens that are normally sequestered from the immune system, and these antigens are capable of provoking an immune reaction in the host, if exposed to the immune system. Several studies have attempted to investigate the presence of tissue-specific antigens in the conceptus.

The presence of embryonic tissue-specific antigens has been demonstrated in a few studies. Wiley and Calarco (1975) prepared antisera to mouse blastocysts which

after absorptions with adult mouse tissues, still reacted weakly in an immunofluorescence assay with zygotes. This reaction increased with each cleavage stage of the embryo to reach a peak reactivity with the 8 to 12-cell stage embryo. The anti-blastocyst serum used in this study also lysed 2 cell- and 8 cell- stage preimplantation embryos in a cytotoxicity assay. In addition, Wiley and Calarco (1975) also raised anti-placental serum which was absorbed with adult mouse tissues and still found to react with mouse eggs, pre-implantation embryos, as well as with the ICM and trophectoderm of early blastocyst outgrowths and trophoblastic components of the definitive placenta but did not react with adult mouse tissues. Such studies have clearly described the presence of tissue-specific antigens on the embryo and placenta (Wiley, 1979).

A variety of other placenta-associated and placenta-specific substances have been described (Klopper, 1980) but most of these proteins await proper characterization. Faulk *et al* (1977) obtained evidence for the presence of trophoblast-specific antigens by preparing purified human trophoblast membranes and then generating heterologous anti-trophoblast antisera which did not cross-react with normal adult tissues.

It is clear therefore that the conceptus expresses some antigens which are tissue-specific; but it is not known whether normal pregnant females recognize these antigens as foreign.

Oncofetal Antigens

Embryos appear to share some cell surface antigens with malignant tissues. Heyner (1980) has suggested a new terminology "neogen" to describe those antigens that are present on both embryonic cells and on tumor cells as well. Neoplastic tissue often appears to regress to a fetal phenotype, with the subsequent production of embryonic antigens like carcinoembryonic antigen (Terry *et al*, 1975), and fetal proteins like alpha fetoprotein (Abelev, 1975), human chorionic gonadotropin (Loke, 1978) and chorionic somatomammotropin (Weintraub and Rosen, 1971). These antigens may pose yet another potentially immunogenic stimulus to the mother.

Edidin *et al* (1971) raised an antiserum to an ascites subline (402AX) of strain 129 teratoma, which after absorption with normal adult tissue still retained its reactivity towards the teratoma and cross-reacted with antigens on unfertilized mouse eggs and

blastocysts. They also found that these oncofetal antigens were restricted to the ICM after implantation. These teratoma antigens were detected on several other mouse tumor lines but were absent from normal mouse cells (Gooding and Edidin, 1974). Similarly, antibodies directed against a teratocarcinoma line could bind to the inner cell mass and to the blastocyst trophectoderm, demonstrating the presence of neogens on the early embryo (Calarco and Banka, 1979).

The F9 antigen (or the F9 group of antigens) is the prototypical neogen and it is expressed by a number of embryonal carcinoma cell lines and also by early embryos in a number of species (Artzt *et al*, 1974). The ICM and the trophectoderm express antigen(s) detectable by anti-F9 antiserum. After implantation the expression of F9 antigen becomes restricted to the embryonic ectoderm, finally disappearing by day 10 of development (Jacob, 1977). The F9 antigen is only one example of a neogen, but a variety of other embryonic antigens apparently exist, which cross-react with tumor-associated antigens and are not expressed by normal, adult somatic cells (for review see Irie *et al*, 1979). Ray and Seshadri (1980) have demonstrated that immunization of animals with homogenates of 14-15 day old embryos induces the development of an anti-tumor antibody response in syngeneic animals. Immunized animals were able to inhibit the growth of a chemically induced fibrosarcoma in tumor-transplantation studies, while spleen cells and sera from immunized animals manifested tumor-killing capabilities in adoptive transfer experiments. Ray *et al* (1982) have also demonstrated that Swiss strain mouse embryos and sarcoma-180 tumors share a common oncofetal antigen. These results were based on immunodiffusion and immunoelectrophoresis experiments performed on antisera made by immunizing rabbits with semi-purified antigen extracts from sarcoma-180 tumor cells and from 12-14 day old syngeneic Swiss mice embryos. Solter and Knowles (1978) have produced a monoclonal antibody that reacts with what they have termed Stage-Specific Embryonic Antigen (SSEA-1) which is expressed on the morula stage ICM. Anti-SSEA-1 antibody reacts with all mouse teratocarcinoma cell lines tested and human teratocarcinoma lines, but not with any other mouse or human cell line tested. It does not react with liver and spleen tissues but appears to react with murine brain and kidney cells. This antigen may not be a true embryo-specific neogen, but Solter and Knowles (1979) suggest that an unspecificied number of intermediary cell types may

exist during embryonic development and that each of these intermediary cell types may express embryonic stage-specific antigens which may differ from those expressed by ancestral cell types and also their final progeny.

It is conceivable that such embryo-specific oncofetal antigens may provide sufficient immunogenic stimuli to the maternal immune system. It is also conceivable that if maternal immunity to such antigens is elicited, then embryonic development may be affected. Very little is known about the incidence and effects of anti-oncofetal sera on pre-implantation development.

In conclusion, it is evident that potentially "foreign" antigens are present on the developing embryo and extra-embryonic tissues. Hence, it can be concluded that a general theory of antigenic immaturity of the conceptus is untenable (Mendenhall, 1976). A question then arises; is the pregnant mother aware of her antigenically foreign fetus ?

E. Maternal Anti-Fetal Reactivity

Many workers have forwarded the hypothesis that the fetal allograft survives because of a decreased maternal immune response during pregnancy (reviewed by Anderson, 1972; Gill and Repetti, 1979). A question that many investigators have tried to address is whether or not there is an impairment in the intrinsic reactivity of maternal lymphocytes during pregnancy. The various parameters that have been studied in man and in the mouse include responses of maternal lymphocytes to phytohemagglutinin (PHA), the capacity of lymphocytes to respond to allogeneic stimuli as measured in the mixed lymphocyte reaction (MLR), maternal B cell responses as assessed by the number of plaque forming cells to sheep red blood cells, the capacity of pregnant animals to reject allogeneic skin grafts, reactivity of lymphocytes to purified protein derivative and the relative numbers of T and B cells in pregnancy. The mass of data now available is confusing, because there are controversies relating to each of the abovementioned parameters (Gill and Repetti, 1979; Gill, 1982). Under optimal conditions there appears to be little or no depression in responses of lymphocytes from pregnant females as compared to the responses of lymphocytes from non-pregnant animals (Carr *et al*, 1973). The inherent immunocompetence of human maternal T cells was demonstrated in the MLR assay by dose response kinetics over a wide range of stimulator and responder

cells; thus there is no evidence for tolerance to paternal histocompatibility antigens (Harrison, 1976a, b). Using the graft versus host reaction (GVHR) in genetically defined strains of mice, Harrison (1976a) has also demonstrated the ability of pregnant mice to mount a successful GVHR against paternal and unrelated cells. These findings effectively eliminate the possibility of inherent T cell hyporeactivity during pregnancy. Despite reports to the contrary, most studies do not show any strong evidence for a consistent effect of pregnancy on the intrinsic reactivity of maternal lymphocytes (Gusdon, 1976; Rocklin *et al*, 1979). Therefore maternal lymphocytes appear to be fully reactive during pregnancy (Gill and Repetti, 1979) and any depression in reactivity is probably due to the activity of pregnancy-associated proteins or hormones.

Immune responses to pregnancy can be discussed under three main categories:

(i) morphologic alterations in lymphoid organs,

(ii) cell-mediated responses, and

(iii) humoral immune responses.

Morphologic Alterations in Lymphoid Organs during Pregnancy

Pregnancy results in certain characteristic changes in maternal lymphoid organs. The maternal immune response to pregnancy has been defined morphologically by the enlargement of para-aortic lymph nodes that drain the uterus in mice (Beer and Billingham, 1971; McLean *et al*, 1974) and in many other species (Gill *et al*, 1979). Maroni and de Souza (1973) studied the thymus, the spleen and the lymph nodes draining the uterus of primiparous mice. The thymus in both syngeneic and allogeneic matings was found to undergo remarkable involution with the decrease in weights in both groups being similar. A gradual and comparable increase was observed in the splenic weights of both syngeneic and allogeneic pregnancies. The lymph nodes draining the uterus undergo remarkable changes in some strains of allogeneically pregnant mice as compared to syngeneically pregnant mice. Draining lymph nodes (DLN) in allogeneic matings was found to be considerably larger and had significantly higher numbers of dividing cells as compared to DLN from syngeneic matings. These workers concluded that the differences observed in the DLN from the syngeneic and allogeneic mating groups may be attributed to the antigenic composition of the conceptus. The extent of stimulation of

the DLN however was not as striking as in a lymph node draining the site of a skin allograft.

Maternal Anti-Fetal Cell-Mediated Immunity

Paternal skin grafts transplanted on to allogeneically mated animals generally fail to be rejected in an accelerated fashion; accelerated rejection of paternal skin grafts would be expected if pregnancy results in maternal sensitization. Multiparity does not result in accelerated graft rejection and there is some evidence to suggest that multiparity results in a prolongation of graft survival (Anderson and Monroe, 1962) suggesting either an absence of effector cells or the operation of immune enhancement during pregnancy (Breyere and Barrett, 1960; Beer *et al*, 1975). However, Chaouat *et al* (1979) have reported the existence, in the spleens of pregnant mice, of cells specifically sensitized to paternal alloantigens. They found that adoptive transfer of spleen cells from allogeneically pregnant mice (and not from syngeneically pregnant mice) rendered these mice capable of rejecting tumor allografts of the paternal strain in a rapid manner. The authors concluded that allopregnant mothers can elaborate cells that are reactive against paternal antigens of the conceptus and that are able to participate in a rejection reaction against cells bearing these antigens.

Rocklin *et al* (1973) observed the production of migration inhibition factor in 50% of the mothers tested, when maternal and corresponding fetal lymphocytes were mixed together. Youtananukorn and Matangkasombut (1972) investigated the presence of cell-mediated immune reactivity against placental antigens. The lymphocytes from all of the 41 primiparous mothers analyzed were found to be primed to placental antigens, in the MIF assay.

In a majority of reports, however, spleens and DLN from allogeneically mated mice have been found to have very little or no cytotoxic cell activity (Clark and McDermott, 1978; Pavia and Stites, 1979). One of the few exceptions is a report by Smith *et al* (1978) who found low levels of non-specific cytotoxicity in 33% of outbred primiparous murine pregnancies. Smith *et al* (1978) also conducted *in vitro* sensitization experiments designed to determine the level of lymphoid cells primed to paternal alloantigens, and found somewhat elevated levels of cytotoxic cells in pregnant females as compared to cells from virgin controls. However, the appearance of cytotoxic cells in

this study was transient.

In addition, Wegmann *et al* (1979c) demonstrated that spleen and mesenteric lymph node cells from pregnant female mice are not primed to paternal alloantigens. Spleen cells from pregnant animals exhibited no more cytotoxicity than normal cells from virgin mice. Maternal lymphoid cells could however be stimulated by *in vitro* exposure to cells bearing the paternal haplotype; thus there is no evidence for an inherent inability of these cells to respond to paternal alloantigens. Wegmann *et al* (1979c) also performed *in vivo* immune elimination studies with target cells and found that allogeneically pregnant females in various stages of gestation (days 5, 11 and 19) were not primed to paternal antigens. These pregnant female mice could however be experimentally primed to paternal alloantigens *in situ*. Wegmann *et al* (1979c) concluded that the presence of an allogeneic fetus generally does not prime the mother to paternal MHC antigens but that this is not due to an inherent incapability of maternal cells to respond, because allogeneically pregnant mice can be readily primed to paternal alloantigens by the injection of paternal strain spleen cells.

Maternal Anti-Fetal Humoral Immunity

Antigens of the fetus that are most immunogenic in the context of humoral immunity are paternally-inherited MHC antigens and fetal red blood cell (RBC) antigens. This contention is supported by evidence for the presence of anti-paternal alloantibodies in pregnant females of many species. Cytotoxic and hemagglutinating antibodies directed at H-2 alloantigens are detectable in pregnant mice (Herzenberg and Gonzales, 1962; Kaliss and Dagg, 1964; Mishell *et al*, 1963). Baines *et al* (1976) reported a substantial increase in the number of specific rosette forming cells in the spleens of multiparous pregnant mice; these responses were specific to paternal MHC and MiH antigens. Voisin and Chaouat (1974) eluted antibodies from murine placentas and found that these antibodies exhibited specificity for paternal strain antigens; the eluted antibodies could bind to paternal strain lymphocytes and had tumor-enhancing capabilities in adoptive transfer experiments. This was the first demonstration of maternal anti-paternal antibodies actually bound to the placenta. However, they did not verify whether these antibodies were directed against paternal MHC antigens or MiH antigens. They also found that a substantial portion of the eluted antibody was of the IgG_1 subclass; IgG_1 antibodies

do not bind complement hence are non-cytotoxic. Bell and Billington (1980) recently reported that the major alloantibody response directed against paternally inherited fetal H antigens in multiparous females is of the IgG_1 subclass with potentially enhancing properties. However, it is well established, at least in humans, that antibodies with cytotoxic activity may be detected in up to 15% of primiparous and 60% of multiparous women (Terasaki *et al*, 1970). Doughty and Gelsthorpe (1974) found an increase in the incidence of cytotoxic antibodies through the first and second pregnancies. It is quite possible that even non-cytotoxic antibodies could pose a threat to the fetus and may act by cross-linking antigenic sites on the cell surface or by promoting opsonization. Anti-paternal HLA-A and HLA-B antibodies are detectable in upto 60% of multiparous women, the incidence and titers of these antibodies increasing with parity. These antibodies are of the leukoagglutinating and cytotoxic variety (Doughty and Gelsthorpe, 1976; Jeannet *et al*, 1977; Revillard *et al*, 1973) and are capable of inhibiting mixed lymphocyte reactions which is indicative of the possible role of these antibodies in preventing reactions between maternal lymphocytes and fetal antigens expressed on placental and fetal cells (Revillard *et al*, 1973).

There is very little evidence to support the contention that high levels of anti-paternal antibodies can harm the fetus. Jeannet *et al* (1977) and Harris and London (1976) suggest that on rare occasions HLA antibodies may leak into the fetus and may then cause disorders such as neonatal thrombocytopenia. It is not possible, on the basis of presently available data, to draw unequivocal conclusions regarding the etiologic relationship between anti-HLA antibodies and fetal abnormalities. Antibodies directed at blood group antigens, of the paternal haplotype have been described in human pregnancies (Takano and Miller, 1972), and erythroblastosis fetalis, caused by the passage of anti-Rh antibodies into an Rh positive fetus, is a well-known phenomenon (Woodrow, 1965).

Antibodies directed at placental tissue-specific antigens have been described by Hulka *et al* (1963).

Evidence that parity induces antibodies reactive with oncofetal antigens is beginning to accumulate. Edidin *et al* (1977) have reported the detection of serum antibodies and cell-mediated immunity to teratocarcinoma antigens in multiparous female

mice. The effect of these antibodies on embryos is not known. Jacob (1979) reported the effects of an antiserum reactive against F9 cells on the cell cleavage of mouse embryos. Divalent anti-F9 antibodies had no obvious adverse effects on pre-implantation development *in vitro*; Fab fragments of this antibody prevented compaction and reversed compaction already underway.

In summary, there appears to be a consensus that except perhaps for a low incidence for cell-mediated immunity, the maternal immune system is not provoked into making consistently strong cellular anti-fetal reactions. However, various other observations have refuted the simplistic notion that the pregnant female is immunologically unaware of the presence of an allogeneic fetus in her uterus. Humoral anti-fetal and anti-placental antibodies are clearly elicited during pregnancy. This dichotomy in maternal immunity - humoral antibody production and the lack of strong cell-mediated effectors - is very intriguing. What can this immune deviation be attributed to? One school of thought maintains that a state of immunosuppression in the mother, brought about by hormones and/or immunosuppressive cells, is responsible for the lack of successful host-versus-graft reactions in pregnancy.

F. Immunoregulation during Pregnancy

One of the simplest explanations that has been put forward to explain the success of the fetal allograft is that the immune system of the pregnant female is *systemically* suppressed by pregnancy-associated hormones; and that as a consequence of this phenomenon the mother is rendered incapable of generating deleterious anti-fetal reactions (Medawar, 1953). Such a generalized non-specific suppression would be conducive to potentially fatal infections but there is no consistent evidence for an increased susceptibility to diseases during pregnancy. Furthermore, a lack of systemic suppression in pregnancy has been clearly demonstrated (Pavia and Stites, 1979).

A multitude of protein and steroid hormones are elaborated by the conceptus during pregnancy and conceivably any of these factors may exercise some immunoregulatory influence on the activity of maternal immune cells during pregnancy. Whether such immunosuppression is exquisitely specific for paternal and fetal antigens or non-specific, and whether it acts locally, is currently an area of intense investigation.

Based on evidence discussed before (see MATERNAL ANTI-FETAL RESPONSES) it is clear that the intrinsic reactivity of maternal lymphocytes is unaltered during pregnancy but it is possible that circulating suppressive factors of a somewhat unspecificed nature may modulate antigen recognition and/or effector cell function in pregnancy. Factors that have been suggested as being naturally-occuring immunosuppressants are human chorionic gonadotropin (hCG), human somatomammotropin (hCS), alpha-fetoprotein (AFP), progesterone, and human placental lactogen (Pavia and Stites, 1979; Gusdon, 1976; Stites *et al*, 1979). hCG and hCS, synthesized by the trophoblast, were considered as strong candidates for immunosuppressive agents (Contractor and Davies, 1973) until it was clearly demonstrated that highly purified preparations of hCG and hCS are not suppressive in MLR assays (Morse *et al*, 1976; Caldwell *et al*, 1975). It was suggested that impurities in preparations of these hormones could account for their putative immunosuppressive activity (Morse *et al*, 1976). Human and murine AFP, a glycoprotein hormone produced during pregnancy by the yolk sac and later by fetal liver have been reported as having an inhibitory effect on certain T cell subsets (Murgita and Tomasi, 1975; Murgita, 1976), but these results have not been substantiated (Sheppard *et al*, 1977).

Stites *et al* (1979) suggest that *local* immunosuppression would ideally prevent the generation of and/or attack by, maternal lymphocytes on placental tissue and thus could contribute to the integrity of the placental barrier necessary for successful gestation. These workers maintain that progesterone, which is an essential hormone of pregnancy in most mammalian species, could be an immunosuppressant in the vicinity of the placenta. The *in vivo* and *in vitro* experiments of Siiteri *et al* (1977) demonstrated that high local concentrations of progesterone are capable of prolonging the survival of skin allografts in rats. Beer and Billingham (1979) were able to extend the survival periods of skin allografts in rats by placing silastic tubes containing progesterone over the grafts. Therefore an argument exists for the possibility of local suppression of the efferent limb of the maternal immunologic reflex arc. However the role of progesterone is suspect because Kitzmiller and Rocklin (1980) reported that progesterone does not suppress lymphocyte reactivity as assessed by the production of lymphokines and target cell responses in the presence of these hormones. These workers therefore dispute that

there is a physiological role for placental steroid hormones as modulators of maternal responsiveness

. There is evidence to support the suggestion that antibodies and immune complexes directed at paternal alloantigens and placental antigens may play an immunoregulatory role. Antibodies in maternal plasma have been found to inhibit MLR and also the release of migration inhibition factor (Rocklin *et al*, 1976); Voisin and Chaouat (1974) eluted antibodies bound to the murine placenta and showed that these antibodies enable normal mice to accept sarcoma allografts of paternal origin. The precise immunogenetic specificities of these antibodies were not conclusively established.

Clark and coworkers (1978, 1980, 1981) have reported a localized impairment of the generation of cytotoxic T lymphocytes (CTL) *in vivo* and *in vitro* in the para-aortic lymph nodes that drain the uterus. This suppression was attributed to the action of mitomycin C-resistant suppressor cells in the DLN of pregnant mice; furthermore, these cells were reported to be absent from the DLN of allogeneically-mated mice that were resorbing their fetuses, suggesting that these cells are probably involved in suppressing anti-fetal reactivity. Clark and McDermott (1980) found that these suppressor cells in the DLN act in a non-specific manner, and may exert their influence in the milieu of the conceptus. The mode of generation and action of these putative suppressor cells is still a matter of speculation. Slapsys and Clarke (1982) have also found similar suppressor cells in the decidua and these cells appear to secrete a soluble suppressor factor which acts in a non-specific manner. These cells may be very important in that they are present in maternal tissue, directly in contact with fetally-derived trophoblast cells.

In summary, it is possible that *local* immunosuppression by hormones or cells or other factors, may play an important role in maintaining the integrity of the placenta and consequently of the fetus. It should be noted that most of these factors have been suggested to inhibit the action of sensitized lymphoid cells in the pregnant mother. Local immunoregulation in the vicinity of the conceptus may contribute to the absence of consistently demonstrable cellular immunity against fetal antigens. However, anti-fetal humoral antibodies are often detected in maternal serum (see Maternal anti-fetal humoral immunity). We may now address the question as to what tissues in the conceptus are responsible for eliciting antibodies in the mother.

G. Fetal-Maternal Cell Trafficking

The cells in the conceptus that are likely to stimulate maternal anti-fetal antibodies are (i) placental cells, and/or (ii) fetal lymphoid cells. Spongiotrophoblast cells and trophoblast giant cells are in direct contact with maternal decidual cells and the trophoblast in hemochorial placentas is bathed in maternal blood and is therefore accessible to immunocompetent maternal cells. It is also conceivable that fetal lymphoid cells may traverse the placenta and may enter maternal circulation where they may stimulate the maternal immune system. It is important to ascertain whether fetal cells can reach the maternal system in sufficient numbers to provide an immunogenic stimulus. In erythroblastosis fetalis, Rh negative mothers are sensitized by their Rh positive fetuses into making an anti-Rh antibody response; some workers conclude that this phenomenon indicates fetal-maternal transfer of RBC during gestation. These responses, however, may well be induced as a result of fetal RBC entering maternal blood during parturition when hemodynamic imbalances between fetal and maternal circulations may cause leakage of cells from the fetus to the mother (Woodrow, 1965). Apparently the proportion of women with fetal RBC in their circulation during pregnancy is not high (5-20%) and this frequency is higher at delivery (5-50%) (Gill, 1977). Small amounts of fetal RBC (Schroder, 1975) and lymphocytes (Desai and Creger, 1963) have been reported to find their way into maternal blood before parturition. Some studies have employed the F. body-quinacrine staining method to scan for putative male cells in maternal blood; the results have been conflicting and as such have not permitted unequivocal conclusions to be drawn from them (Gill and Repetti, 1979). Herzenberg *et al* (1979) employed elegant cell sorting techniques to examine maternal blood for the presence of fetal cells by using immunogenetic and cytogenetic criteria; they reported the presence of fetal cells in maternal circulation during human pregnancy. There is no direct evidence for the transfer of fetal cells into maternal blood in *murine* pregnancy. It must be emphasized that the amount of immunogenic material (i.e., the number of antigen-bearing cells) entering maternal circulation is far more important than the mere presence of some fetal lymphocytes in maternal blood. It has not been possible to ascertain as yet whether fetal cells coming into contact with maternal circulation do constitute an adequate immunogenic stimulus.

Trophoblast cells frequently detach from the placenta and move into the maternal blood stream; it has been documented that up to one gram of trophoblastic tissue per day may enter the human blood stream, by a phenomenon termed "trophoblastic deportation" (Ikle, 1964). These cells may elicit maternal responses, provided they express antigens foreign to the mother.

Thus the bulk of the evidence argues against significant transfer of murine fetal cells into the mother.

H. The Placenta as a Cell Filter

The exclusion of maternal lymphoid cells by the placenta could be one of the mechanisms by which the fetal allograft is prevented from rejection. Even though cell-mediated anti-fetal reactions are infrequent in pregnancy, in the event such reactions are generated are these sensitized cells capable of reaching the fetus? There have been scattered reports claiming that a significant amount of maternal cells do reach the murine fetus (Tuffrey *et al*, 1969; Barnes and Tuffrey, 1971); these findings have not been substantiated by other workers (Billington *et al*, 1969; Adinolfi, 1975). Beer and Billingham (1973) reported the runting of neonates caused by a putative transfer of sensitized lymphoid cells from the mother to the fetus; these results have not been confirmed in an extensive series of experiments (cited by Billington, 1976). A number of studies have clearly demonstrated that fetuses develop normally despite the mother being strongly sensitized to fetal antigens by paternal skin grafting (Medawar and Sparrow, 1956; Heslop *et al*, 1954; Lanman *et al*, 1962). Wegmann *et al* (1979c) primed pregnant mice to paternal antigens and found that the fetuses in these sensitized mothers were unharmed. There is therefore no direct evidence of any significant transfer of maternal lymphocytes to the fetal circulation (Adinolfi, 1975). Thus, maternal lymphocytes do not appear to traverse the placenta in significant numbers and it is highly likely that the placental barrier prevents the access of all but a few maternal lymphocytes into the fetus.

I. The Placenta as an Antibody Filter

A question that has remained unresolved for a long time and that is central to this thesis relates to the intriguing survival of the allogeneic fetus despite the presence of anti-fetal antibodies in the mother. Human and animal experiments have provided evidence for the presence of cytotoxic antibodies directed at fetal (paternal) antigens during pregnancy (as discussed in Section E). The density of H antigens on most fetal tissues appears to be sufficient for immune attack (Edidin, 1972) and it is therefore conceivable that these potentially deleterious antibodies may harm the fetus if they enter the fetal circulation. Thus, it seems necessary that the intervening placenta act as a filter by trapping such antibody. At the same time, it is well established that the fetus depends on maternal immunological experience i.e., her antibodies, until such time that its own immune system is functional. Hence the placenta would have to act as a selective immunoabsorbent by absorbing those antibodies which have their corresponding antigens expressed on the placenta and at the same time transporting those antibodies that are needed for protection of the fetus.

Swinburne (1970) suggested that maternal antibodies directed at histocompatibility antigens of the fetus are absorbed by placental tissue antigens. He also suggested that antigen-antibody complexes are also filtered out and eliminated by the placenta as fast as they are formed. This hypothesis has been reiterated a few times by others who have suggested that the possession of an array of specific antigenic determinants by the placenta may enable this tissue to serve as a specific immunoabsorbent (Billington and Jenkinson, 1975; Sellens *et al*, 1978). A review of the relevant literature indicates that there is little evidence to support this hypothesis. In humans, anti-paternal HLA antibodies detectable in maternal circulation are not found in the fetal blood if the fetus bears the same haplotype; these antibodies are instead found absorbed to the placenta. However, if the fetus lacks the appropriate antigens, these antibodies are not found in placental eluates, but are detectable in cord blood (Tongio *et al*, 1975; Doughty and Gelsthorpe, 1976). These findings suggest that anti-fetal antibodies are absent from fetal blood due to a sequestration of such antibodies in the placenta. However, these results are not conclusive because the lack of anti-HLA antibodies in fetal blood, may simply be due to the absorption of these antibodies by

fetal tissues. Wegmann *et al* (1979a) found that radiolabeled anti-fetal H-2 antibody was selectively absorbed by the placenta when the fetus bears the appropriate target antigen; this finding lends credence to the idea that the placenta may serve as a paternal antigen-bearing immunoabsorbent barrier between the maternal immune system and the semi-allogeneic fetus.

In the human placenta, paternal HLA antigen-bearing cells in the chorionic villi may provide an immunoabsorbent layer even if the human syncytiotrophoblast is indeed devoid of HLA antigens. McIntyre and Faulk (1979) speculate that the chorionic villi of the human placenta may serve as an antibody filter for a variety of antibodies including anti-Gm and anti-oncofetal specificities.

It thus seems likely that the placenta may provide an immunologic sanctuary for the fetus during gestation.

II. MATERIALS AND METHODS

Mice

Inbred mice of strains BALB/cCR (H-2*d*), C3H/HeJ (H-2*k*) and C3H.A (H-2*a*) were obtained from the Small Animal Breeding Program of the University of Alberta, Edmonton, Alta. The H-2 haplotypes of these strains are shown schematically in Table 1. In these studies the syngeneic matings were BALB x BALB (H-2K*d* x H-2K*d*) and C3H.A x C3H.A (H-2D*d* x H-2D*d*); the allogeneic matings were BALB x C3H/HeJ (H-2K*d* x H-2K*k*) and C3H.A x C3H/HeJ (H-2D*d* x H-2D*k*). Pregnancies were timed by checking for vaginal plugs and the presence of a plug was used as a criterion to determine day zero of pregnancy.

Antibodies

Three different monoclonal antibodies were used in this study. Anti-H-2K*k* antibody of IgG2a subclass was produced by the hybridoma #11-4.1, available through the Salk Cell Distribution Center, La Jolla, California. It reacts with cells bearing the H-2K determinant from H-2 haplotypes k, r, p and q, but not d, b, f or s (Oi *et al*, 1978). Anti-H-2D*k*, also an IgG2a antibody, was produced by the hybridoma #15-5-5S, (a generous gift of Dr. D.H. Sachs). This antibody is directed against the H-2D*k* determinant, but exhibits cross reactivity to the H-2K*d* determinant and to the H-2*f* haplotype antigens (Ozato *et al*, 1980]. Anti-I-A*k* antibody (IgG2a], produced by hybridoma #10-3.6, (Salk Cell Distribution Center, California) is reactive to cells of the f, k, r and s haplotypes, but not with b, d, p or q haplotypes and this pattern of reactivity corresponds to specificity Ia.17 (Oi *et al*, 1978]. The specificities of these antibodies are shown schematically in Table 1. Hybridomas were grown in pristane-primed BALB/cCR mice; the monoclonal antibodies were obtained from these mice in ascites form.

Testing of Anti-H-2 Antibodies

Every batch of ascites fluid was tested in an *in vitro* competitive binding assay with the appropriate ^{125}I-labeled antibody. The method used for radiolabeling antibodies is described in a following section.2 Single cell suspensions were prepared by first mincing spleens in Leibovitz medium (Grand Island Biological Co., New York, NY) and then gently

TABLE 1
H-2 haplotypes of mouse strains and the regions of the H-2 complex recognized by the antibodies used in these studies.

STRAIN	HAPLOTYPE	K	I-A	I-E	D
BALB/cCr	H-2*d*	d	d	d	d
C3H/HeJ	H-2*k*	k	k	k	k
C3H.A	H-2*a*	k	k	k	d
ANTIBODIES*					
Anti-H-2K*k*		k	–	–	–
Anti-H-2D*k*		d	–	–	k
Anti-I-A*k*			k		

* See text for cross-reactivities.

pressing the tissue through 60 mesh steel screens. The debris and cell clumps were removed by allowing them to settle for 10 minutes. The remaining cells were then washed twice with Leibovitz medium by centrifugation at 500 xg for 10 minutes. The cells were resuspended in the same medium.

10,000 cpm of the radiolabeled antibody preparation was mixed with varying amounts of the ascites [from 0.6 to 20 microliters (*ul*)] and added to 10^7 spleen cells suspended in 0.5 milliliters (ml) of the medium. These mixtures were incubated at 4°C for 1 hour and the cells then washed thrice in Leibovitz medium. The residual radioactivity in the pellets was measured in a Beckman Gamma 300 counter (Beckman Instruments Inc., Irvine, CA) and the 50% inhibiting dose of the test ascites was compared with a standard batch of ascites of known reactivity. New batches of ascites containing antibody were always compared to the standard reference batch.

Preparation of F(ab')$_2$ fragments of immunoglobulins

F(ab')$_2$ fragments were prepared by pepsin digestion of IgG. 250 micrograms (*ug*) of pepsin (Sigma Chemical Co., St. Louis, Mo.) were added to 3 ml of 0.15M sodium acetate buffer at pH 4.6, containing 15 mg of the monoclonal antibody. This mixture was incubated for 24 hours at 37°C to allow digestion to occur, and the reaction was stopped by adjusting the pH to 8.0 with Tris base (Nissonoff *et al*, 1960). Undigested immunoglobulins and Fc fragments were removed by Protein-A sepharose chromatography. The F(ab')$_2$ was generously prepared by Dr. B. Singh.

Purification of antibodies from ascites

Antibodies in ascites fluid were purified by Protein A-Sepharose chromatography. A 10 ml Protein A-Sepharose (Pharmacia, Sweden) gel column was prepared and washed thoroughly with 30 ml of phosphate buffered saline (PBS), pH 7.2. Five ml of ascites fluid was passed through the column. The column was then washed with 30 ml of PBS, after which the effluent contained no more detectable protein. Thirty ml of 1M acetic acid was passed through the column to elute the adsorbed immunoglobulins. The pH of the eluted solution was adjusted to pH 7 with Tris base. This purified solution of immunoglobulins was dialyzed against 3 changes of PBS pH 7.2, at 4°C.

The $F(ab')_2$ preparation was also purified by Protein A chromatography. Fc fragments were absorbed by the protein A gel, while $F(ab')_2$ fragments were obtained in the eluent.

Radioiodination of immunoglobulins

^{125}I labeling of antibodies was performed by the chloramine T method of Greenwood *et al* (1963), at a molar ratio of one iodine atom to one IgG molecule. 5 millicuries of radiolabeled sodium iodide (NEN Canada, Lachine, Que.) was added to a 5 milligram (mg) solution of the immunoglobulins in PBS. Fifty *u*ls of chloramine T was added to the mixture and the oxidation reaction allowed to continue for 2 minutes at room temperature. Further oxidation was stopped by neutralizing the mixture with 50 *u*l of sodium metabisulfite. Ten *u*ls of 1% potassium iodide was added and the solution dialyzed against saline to remove free iodine.

Intrinsic radiolabeling of immunoglobulins

Anti-H-2K*k* antibody was intrinsically labeled with ^{3}H-leucine (for autoradiography) and with ^{35}S-methionine (for the "fate" studies described below). 11-4.1 hybridoma cells were grown *in vitro* in RPMI medium [Grand Island Biological Co., New York, NY] and then washed thrice in leucine-free minimum essential medium (or methionine-free MEM in the case of ^{35}S-methionine labeling) (Grand Island Biological Co.). The cells were then suspended in 6 ml of leucine-free MEM (or ^{35}S-methionine-free MEM) at a density of 5 x 10^6 cells per ml. One millicurie of ^{3}H-leucine (or ^{35}S-methionine) (NEN Canada) was added to the cell suspension and the cells incubated for 3 hours at 37°C with intermittent shaking. After 3 hours, the cell suspension was chilled and cold RPMI added; the cells were spun down at 500 xg for 5 minutes and the supernatant containing ^{3}H-labeled anti-H-2K*k* was dialyzed against saline for 48 hours at 4°C to remove free leucine (or free methionine).

Purification of ^{125}I-labeled antibodies

^{125}I-labeled antibodies were purified by absorption-elution on glutaraldehyde-fixed C3H/HeJ spleen cells. 10^9 spleen cells at a concentration of 2 x 10^7 cells per ml in

Leibovitz medium were mixed with an equal volume of 0.25% glutaraldehyde with continuous stirring over a period of 5 minutes. The cells were then washed twice in medium containing 0.1M glycine. The radiolabeled antibody preparation was added to the cell pellet and then incubated for 1 hour at 4°C. The cells were washed twice with Leibovitz medium. The antibodies that were bound to the cells were eluted by adjusting the pH to 3.2 with 0.1M acetic acid and centrifuging the mixture immediately. The pH of the supernatant was restored to 7.3 by adding 3 mls of 0.1M Tris buffer to the supernatant. This supernatant, consisting of specific ^{125}I-labeled antibody, was then tested in a quantitative absorption assay.

Testing of radiolabeled antibodies

Radiolabeled antibodies were assessed for specific activity by comparing the binding of the antibodies to target cells (C3H/HeJ) and the appropriate control spleen cells (BALB/cCR cells in the case of anti-H-2K*k* and anti-I-A*k* antibodies; and C3H.A cells in the case of anti-H-2D antibody). Single spleen cell suspensions were prepared and then suspended in Leibovitz medium (containing 1% bovine serum albumin, Sigma Chemical Co., St. Louis, Mo.) in concentrations ranging from 10^7 to 4 x 10^8 cells per tube. 10^5 cpm of the labeled antibody were mixed with the cells and incubated for 1 hour at 4°C. The cells were pelleted and washed twice. The radioactivity in the resultant cell pellets was measured in a gamma counter. The binding of ^{125}I-antibody to target and control cells was compared in order to determine the specific activity of the labeled antibody preparation.

Processing of fetal and placental tissues for measurement of radioactivity

In many of the studies described herein, it was necessary to estimate the binding to placentas and fetuses, of ^{125}I-antibodies injected into pregnant mice. The placentas and fetuses from each animal were removed and the placentas carefully separated from the fetuses. The placentas and fetuses were collected separately in a solution of 1% heparin [v/v] in PBS and weighed. The placentas were washed thoroughly in heparin-PBS and the tissues ground in a glass homogenizer. The homogenate was centrifuged and the resulting pellet washed twice in PBS. Fetuses were washed, then minced into smaller pieces. The uptake of antibody by the tissues was estimated by measuring the radioactivity of the

tissues in a gamma counter.

Localization of paternally-derived H-2K antigens on the placenta by autoradiography

In order to localize the binding of radiolabeled anti-paternal H-2K antibody on the placenta, autoradiography was performed on placentas from target (*d*x*k*) and control (*d*x*d*) pregnant mice injected with the radiolabeled antibody. The ^{125}I-antibody or the ^{3}H-antibody (10^{6} cpm per animal) was injected into target and control pregnant mice on days 10, 13 and 17 of gestation. Mice were anesthetized with Penthrane (Abbott Labs Ltd., Chicago, IL) at 4 hour and 8 hour intervals after injection and then perfused with 100 ml of 1% heparin-saline through the right ventricle of the heart. The liver and the placentas were blanched after this procedure, indicating that the blood vessels in these organs had been perfused with saline. The purpose of this step was to wash out the unadsorbed radiolabeled antibodies from the placental circulation, leaving the bound antibodies intact. The placentas were then dissected from the uterus with the decidua attached and cut into wedges; the tissues were then fixed in 0.35% glutaraldehyde in 0.1M PBS and then embedded in glycol methacrylate. 2 *u*m sections were prepared and then coated with Kodak NTB 3 nuclear track emulsion. After exposure at 4°C for 4 to 25 weeks, the autoradiograms were developed in Kodak D-19 developer, and stained with Gill's hematoxylin and Lee's methylene blue-basic fuchsin.

In order to test for the possibility of non-specific Fc binding, the binding of ^{125}I-F(ab')$_2$ anti-H-2K*k* was also examined. 10^{6} cpm of the F(ab')$_2$ preparation were injected i.v. into *d* x *d* and *d* x *k* pregnant mice and the placentas excised 4 and 8 hours later. These placentas were processed for autoradiography as described. The distribution of the label in target and control syngeneic placentas was examined. This work was performed in collaboration with Drs. D.J. Anderson and B.A. Sandow, of the Oregon Regional Primate Research Center, Beaverton, Oregon.

Fetectomy

BALB/cCR mice mated with BALB/cCR or C3H/HeJ males were fetectomized on day 11 of gestation. The mice were anesthetized with penthrane and then a midline incision made

over the peritoneal cavity. The uterine horn was exteriorized and a small hole made on the uterine surface directly opposite to the placental attachment site using two forceps. The fetus was removed through the hole and the umbilical cord severed, leaving the placentas intact. The pressure inside the uterus was usually high enough to cause the fetus to be extruded without further manipulation once the hole was made on the uterine wall.

Depending on the experiment, either all the fetuses were removed or only the fetuses in one horn were removed leaving the other uterine horn intact, as a control. Finally the uterus was replaced, and two wound clips were used to close the incision. The whole operation was performed under sterile conditions.

Fate of anti-paternal H-2 antibody bound to the placenta

10^6 cpm of ^{125}I-anti-H-2K*k* antibody were injected into *d* x*d* and *d* x *k* pregnant females and the mice anesthetized at different time intervals (from 1 hour to 12 hours) after antibody injection. The animals were perfused and the placentas excised. Placental tissues from each animal were homogenized in a glass homogenizer to obtain single cell suspensions. These cells were washed with 1% heparin-PBS twice and then resuspended in 3 ml of distilled water, containing 3 mM phenyl methyl sulphonyl fluoride [PMSF] added to prevent the degradation of immunoglobulins by enzymes that could be released by the cell-rupturing procedures described below.

The cells were then subjected to ultrasonication at 4°C in order to rupture the cells and the cytoplasmic contents collected for analysis of IgG breakdown products. Three 10 second periods of sonication were sufficient to rupture a majority of the cells using a Biosonik Sonicator [Bronwill VWR Scientific, San Francisco, CA]. The resultant mixture was centrifuged at 500 xg for 10 minutes to remove the cell debris and then at 100,000 xg for 60 minutes to remove the cell membranes. The supernatant containing the cytoplasmic contents of the cells was then analyzed for breakdown products of IgG by gel chromatography.

The cytoplasmic extracts were fractionated on Sephacryl-200 [Pharmacia, Sweden] A 100 cm x1.2 cm Sephacryl-200 column was prepared and equilibrated with PBS, pH 7.2. The column was calibrated with marker proteins of different molecular weights. These marker proteins were IgG (molecular weight=150,000), bovine serum

albumin (70,000) and trypsin (24,000). 10^5 cpm of the samples to be analyzed were passed through this column and 1 ml eluate fractions were collected. The radioactivity of these fractions were measured in a gamma counter.

In order to examine the processing of the anti-H-2Kk antibody by other tissues, the radiolabeled antibody was injected into BALB/cCR and (BALB x C3H)F_1 female mice and the livers excised after different time periods. These tissues were then processed as descibed above and the breakdown products analyzed by gel filtration.

III. RESULTS

A. Testing of Anti-H-2 Antibodies

Every batch of ascites fluid containing anti-H-2 or anti-Ia antibodies was tested in a competitive inhibition assay and compared to the inhibition of binding of ^{125}I-anti-H-2 or anti-Ia antibodies to target C3H/HeJ spleen cells, by a reference batch of the same antibody (Figure 3). Inhibition curves were plotted for the test batch and for the reference batch of the antibody and the 50% inhibiting dose (defined as the amount of unlabeled antibody required to inhibit the binding of 10,000 cpm of the labeled antibody) calculated. As can be seen in Figure 3, 6 *u*ls of the test ascites are equivalent to about 3.5 *u*ls of the standard reference batch of the same antibody, by comparing the 50% inhibiting dose.

B. Testing of Radiolabeled Antibodies

Radiolabeled antibodies were tested in a quantitative absorption assay, wherein the binding of labeled antibodies to target spleen cells, was compared to its binding to control cells. The results of a typical assay for ^{125}I-anti-H-2K*k* are presented in Figure 4. As shown in this figure, 70% of the labeled antibodies bound to the target cells (4 x 10^6 cells), while the binding of this antibody to the non-target control cells was only 5%, indicating that the specific activity of the antibody was 65%.

The specific activities of the different batches of the ^{125}I-labeled antibodies (anti-H-2K*k*, anti-H-2D*k* and anti-I-A*k*) ranged between 56% and 68%. The intrinsically ^{3}H-labeled anti-H-2K*k* and ^{35}S-labeled anti-H-2K had specific activities of 38% and 44% respectively.

C. Evidence for the Placental Immunoabsorbent Model

Antibodies directed at paternally-derived fetal alloantigens are elaborated during pregnancy. The placenta has been postulated to protect the fetus by absorbing antibodies directed at fetal antigens. This series of experiments was performed to address the question of whether the placenta can indeed serve as an immunoabsorbent between the

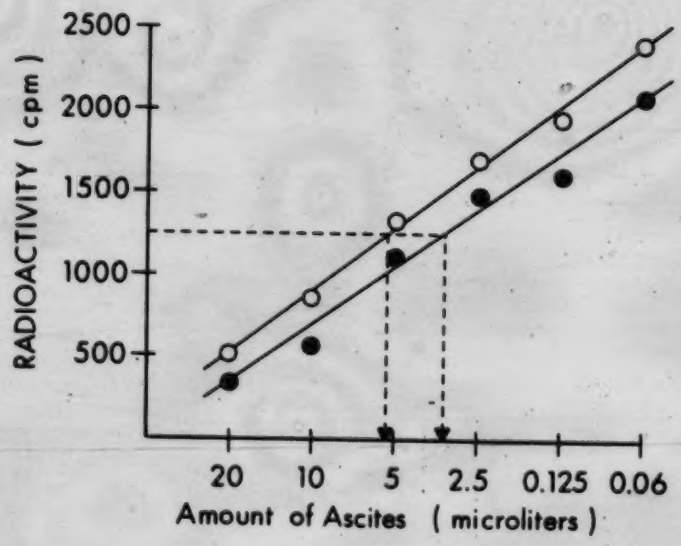


Figure 3 – Competitive inhibition assay to assess the antibody activity of ascites. The amounts of a standard batch of antibody (●) and test ascites (O) required to inhibit the binding of labeled antibody by 50% were determined.

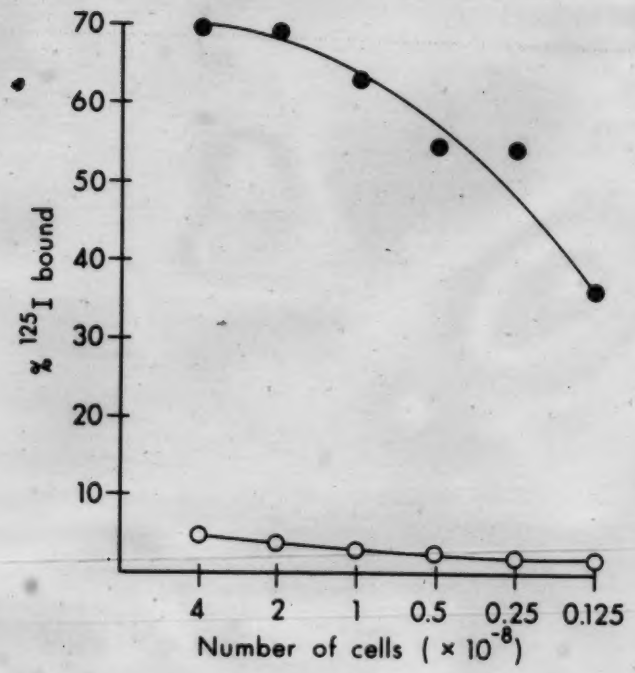


Figure 4 – Quantitative absorption of radiolabeled anti-H-2*k* antibody by target (H-2*k*) spleen cells (●) and control (H-2*d*) spleen cells (○).

maternal and fetal circulations. If the placenta can serve as a barrier to the entry of anti-paternal H-2*k* antibodies into the fetus, then anti-paternal antibodies injected into maternal circulation would be expected to reach the *d* x *d* fetus in higher concentrations than the *d* x *k* fetus, as these antibodies would be absorbed by the relevant antigens on the *d* x *k* placenta. To evaluate this prediction, ^{125}I-antibodies were injected into control (*d* x *d*) and target (*d* x *k*) pregnant animals and the uptake of antibodies by the control and target placentas and fetuses were compared. 2 x 10^5 cpm of ^{125}I-labeled anti-H-2K*k* antibody were injected intravenously (i.v.) into pregnant mice on day 13 of gestation. The animals were sacrificed at various time points thereafter and the placentas and fetuses excised. The values obtained were expressed as cpm per fetus and cpm per placenta.

The amount of antibody binding to target placentas is markedly higher than that binding to control placentas during the first 48 hours after injection (Figure 5). Conversely, the amount of labeled material in control fetuses is higher than that in the target antigen-bearing fetuses. These results are consistent with the postulate that the allogeneic placenta selectively absorbs the anti-paternal H-2 antibody, allowing only low levels of antibody to get through into the fetus.

It is possible that Fc receptors on placental cells may influence the binding properties and kinetics of anti-paternal antibodies in the placenta. In order to verify whether the Fc receptors in the placenta had an influence on the kinetics of antibody uptake and loss in the placenta, ^{125}I-labeled F(ab')$_2$ anti-H-2K*k* was injected into 13-day pregnant mice and the radioactivity in the intact placentas and fetuses measured at various time points after injection, as was done for the IgG antibody. The immunoabsorbent effect of the placenta is also seen with the F(ab')$_2$ antibody; the level of anti-H-2 antibody is higher in the fetus, if it does not bear the relevant target antigen (Figure 6). The results also indicate that the peak of binding is quite similar to that seen with the IgG molecule, but F(ab')$_2$ disappears more quickly from the placenta. This suggests that the breakdown of the F(ab')$_2$ anti-H-2K in the placenta might in some way be different from the breakdown of the intact IgG antibody, even though the uptake patterns are similar.

(&5line, Chap('Time Course of Placental Antibody Binding')> Previous work done in this

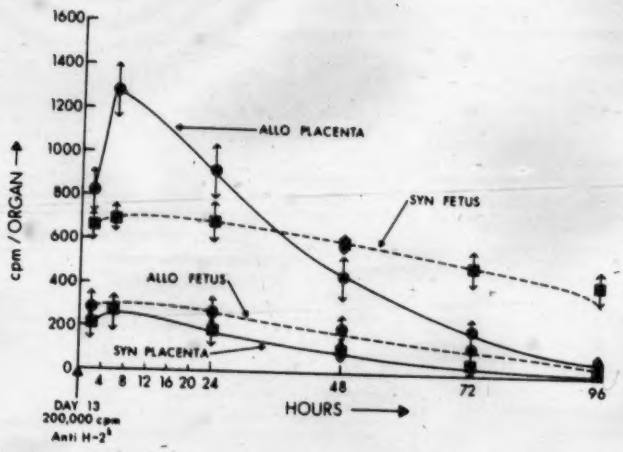


Figure 5 – Evidence for the placental immunoabsorbent model. The relationship between the amounts of ^{125}I present at various time periods in control and target placentas and fetuses after a single pulse of ^{125}I–anti–H–2K*k* antibody. Each point represents the radioactivity cpm/organ ±S.D. (●——● target *d*x*k* placenta; ■——■ control *d*x*d* placenta; ●- - - -● target fetus; ■- - - -■ control fetus)

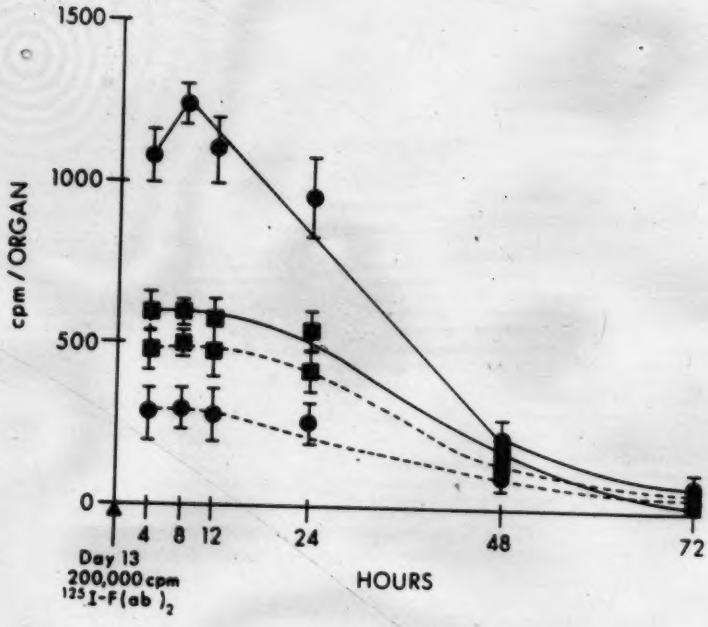


Figure 6 – Testing of immunoabsorbent antibody kinetics using $F(ab)_2$ anti–H–2 antibody. The relationship between the amount of ^{125}I present at various time points after a single pulse of ^{125}I–$F(ab)_2$ anti–H–2K*k*. (●——● target *d*x*k* placenta; ■——■ control *d*x*d* placenta; ●- - - -● target fetus; ■- - - -■ control fetus)

laboratory has established that the allogeneic (*d* x*k*) placenta expresses paternally-inherited H-2*k* antigens and that these antigens are accessible to maternal circulation (Wegmann *et al*, 1979). It was considered feasible to enumerate the paternal H-2 antigens on the placenta by using this *in vivo* procedure. For this purpose it was necessary to first determine the time interval required for optimal binding of antibodies to the placenta *in vivo*.

In order to determine the optimal time period required for anti-paternal antibody to bind to the placenta, a time course experiment was performed. 2 x 10^5 cpm of the ^{125}I-anti-H-2K*k* antibody was injected i.v. into *d* x *d* and *d* x *k* pregnant mice on days 10, 13 and 17 of gestation. The placentas were excised and collected at different time periods after the injection of antibodies. The placental tissues were processed as described in MATERIALS AND METHODS and the radioactivity of the homogenates then measured in a gamma counter.

Figure 7A, B and C depict the binding of ^{125}I-anti-paternal antibodies to target and control placentas as a function of time on days 10, 13 and 17 respectively. It is evident that maximal binding occurs 6 hours after the injection of antibody, and remains stable for a few hours, after which the ^{125}I content of the placentas decreases steadily with time. This finding is consistent for days 10, 13 and 17 of gestation.

In all subsequent studies of placental antibody uptake, the placentas were removed 6 hours after the injection of ^{125}I-antibody.

D. Quantitation of Paternal H-2K Antigens on the Placenta

This series of experiments was performed with the objective of enumerating the paternally-derived H-2 antigens on the allogeneic placenta. This was accomplished by first ascertaining the absorptive capacity of the placenta for anti-paternal antibodies *in vivo*, by performing dose response experiments. It was then possible to determine the maximal absorptive capacity of the placenta and to calculate the density of paternal H-2K antigens on the placenta.

Graded amounts of the ^{125}I-antibody were injected into BALB females bred with BALB males or with C3H/HeJ males. The placentas were removed 6 hours post-injection, based on the finding that optimal binding of anti-paternal antibody occurs 6 hours after

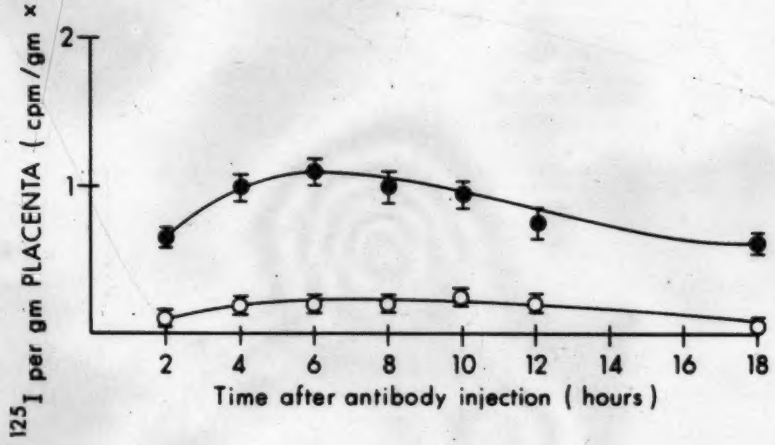


Figure 7A – Time course of the binding of anti-H-2K*k* antibody to target (●) and control (○) placentas on day 10 of gestation.

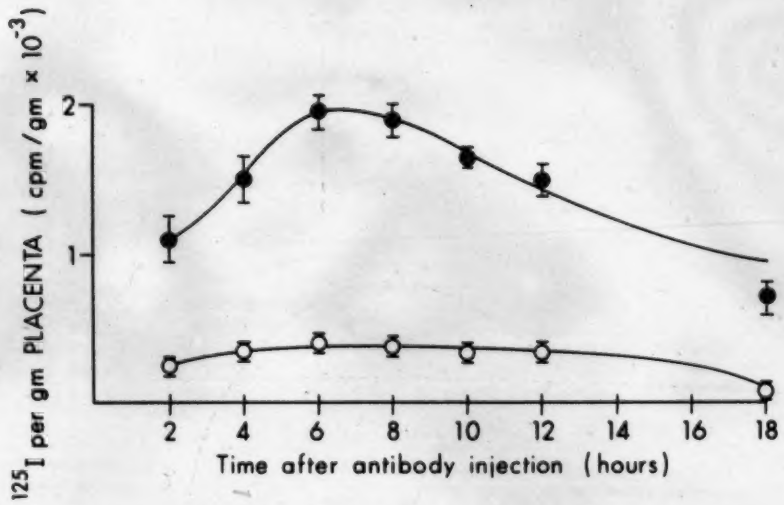


Figure 7B – Time course of the binding of anti-H-2K*k* antibody to target (●) and control (○) placentas on day 13 of gestation.

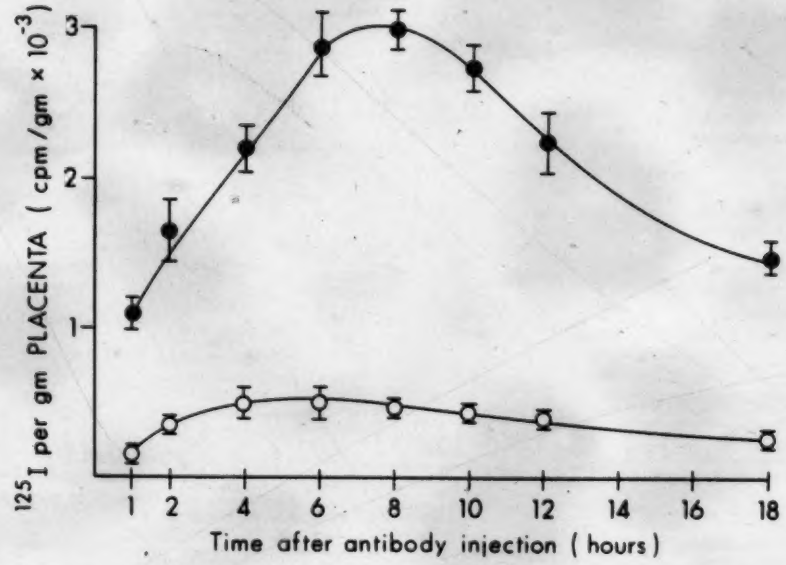


Figure 7C – Time course of the binding of anti-H-2K*k* antibody to target (●) and control (○) placentas on day 17 of gestation.

injection of antibody (Figure 7). The tissues were processed for gamma counting.

The uptake of labeled antibody by the target placentas substantially exceeded the uptake of the same antibody by control placentas on day 10 (Figure 8A), day 13 (Figure 8B) and day 17 (Figure 8C) of gestation. There was an increase in the amount of antibodies bound by the placenta as gestation proceeded – the older placentas showing more uptake per gram placental tissue. This is depicted in Figure 9, where the *specific* uptake of antibody (uptake by *d* x *k* placentas minus uptake by *d* x *d* placentas) is plotted as a function of the amount of radiolabeled antibody injected. The differences, in dose responses at different stages of gestation are reflective of the increase in density of paternal H-2 antigens on the placenta during its development. In order to estimate the density of paternal H-2K antigens on the placenta, the dose response curves (Figure 9) were plotted as double reciprocal curves (Figure 10). The intersect of each of these double reciprocal curves on the Y-axis theoretically represents the reciprocal of the amount of antibody that would bind to the placenta if all the H-2K sites on the placenta were to be saturated with antibody following the injection of an infinite amount of antibody. The amounts of antibody bound at saturation by the placentas on days 10, 13 and 17 were thus estimated to be 6,500 cpm/gm, 11,500 cpm/gm and 14,300 cpm/gm respectively. As 10,000 cpm of the labeled antibody preparation corresponds to 1 *u*g of antibody protein, the uptake of antibody at saturation is 0.65 *u*g/gm (day 10), 1.15 *u*g/gm (day 13) and 1.43 *u*g/gm (day 17) placental tissue. Since the molecular weight of IgG is 150,000 daltons, these values correspond to 4.2×10^{-12}, 7.5×10^{-12} and 9.3×10^{-12} mol antibody molecules bound per gram placenta, or 5.0×10^{12} antibody molecules per gram placenta on day 10, 8.9×10^{12} on day 13 and 11.1×10^{12} on day 17 of gestation. Standard errors of these estimates were determined by calculating the standard errors of intercepts of the double reciprocal curves. The estimate of the number of H-2K determinants on day 10 is significantly different from those on day 13 (p 0.0025) and on day 17 (p 0.001). However, even though there is an apparent increase in the immunoabsorptive capacity from day 13 to day 17, the values are not significant when examined statistically. The experiments were repeated and the difference in immunoabsorptive capacities of day 13 and day 17 placentas was found to persist.

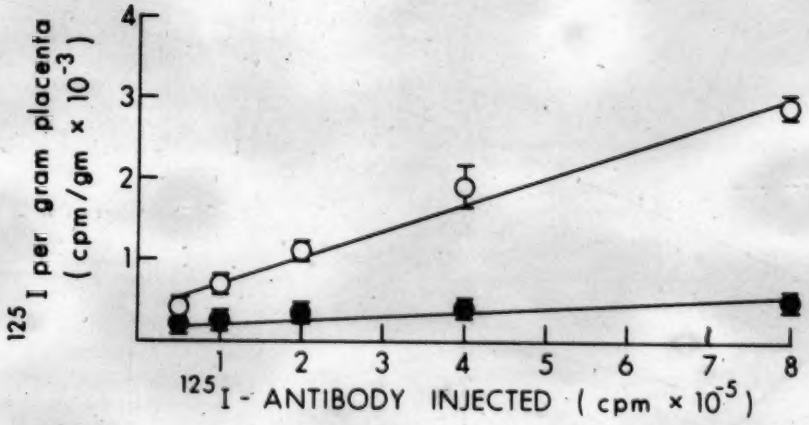


Figure 8A – Binding of ^{125}I–anti–H–2K*k* antibody to target *d*x*k* (○) and *d*x*d* (●) placentas on day 10 of gestation. Each point represents the mean ±S.D. of the cpm per gram of antibody bound to the placenta.

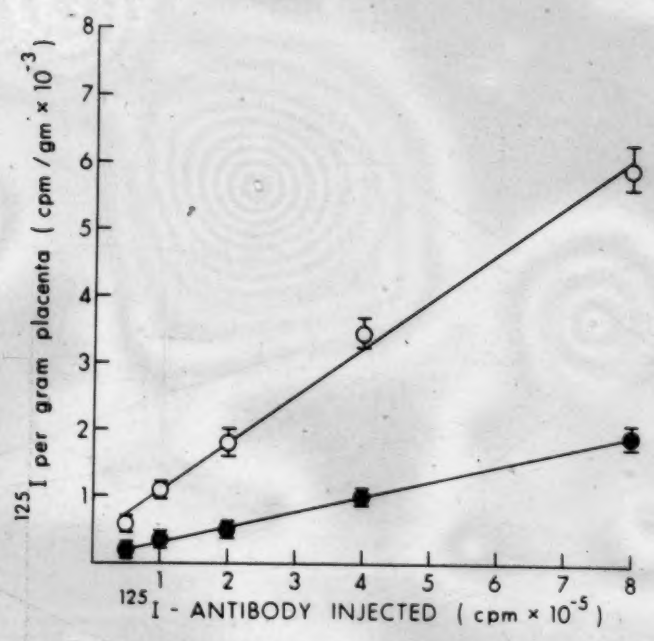


Figure 8B – Binding of ^{125}I–anti–H–2K*k* antibody to target (○) and control (●) placentas on day 13 of gestation.

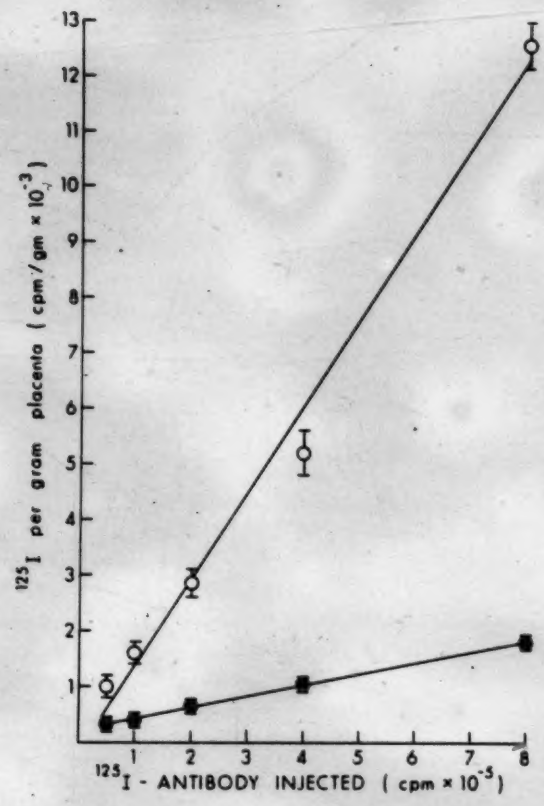


Figure 8C – Binding of ^{125}I–anti–H–2K*k* antibody to target (○) and control (●) placentas on day 17 of gestation.

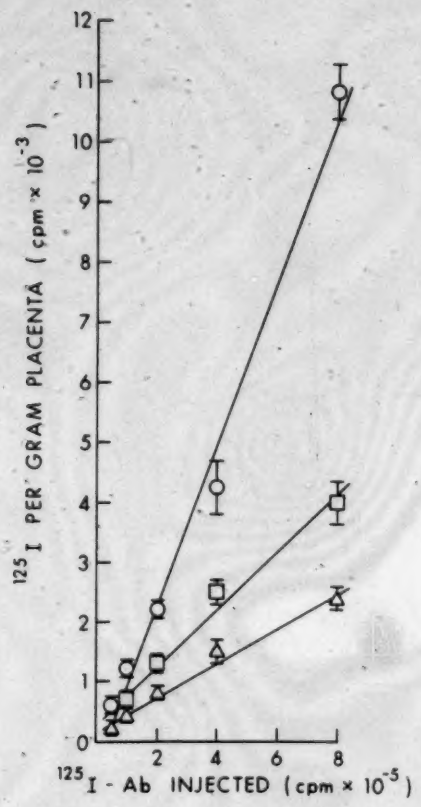


Figure 9 – Comparison of the specific binding of antibody to target and control placentas at different stages of gestation. The curves represent dose responses on day 10 (Δ), day 13 (□) and day 17 (○) of gestation.

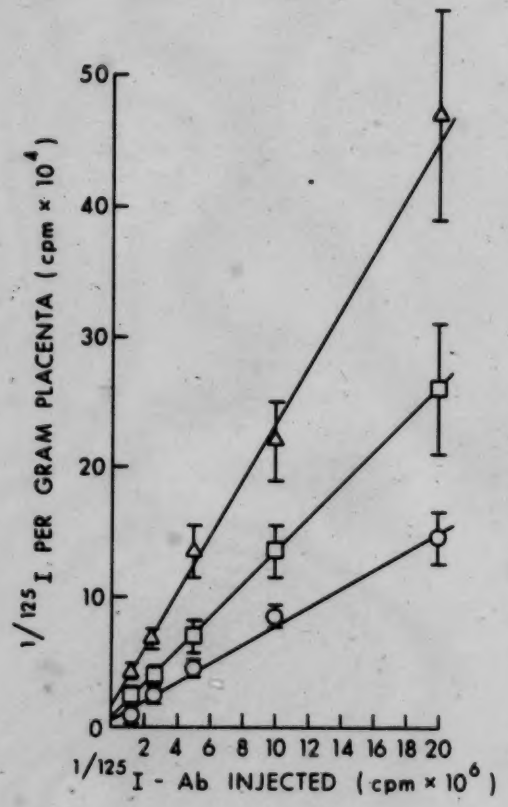


Figure 10 – Determination of the placental immunoabsorbent capacity for anti-paternal H-2K antibody. The intercepts of the double reciprocal curves representing dose responses on day 10 (Δ), day 13 (□) and day 17 (○) were used to calculate the placental capacity for anti-paternal H-2K antibody.

It is clear that the estimated density of paternal H-2K antigens per gram tissue, increases throughout gestation (Figure 11).

E. Quantitation of Paternal H-2D Antigens on the Placenta

The density of paternal H-2D determinants on the allogeneic placenta was estimated in the same manner as described for H-2K antigens above, using C3H.A x C3H.A as the control and C3H.A x C3H/HeJ as the target pregnancies. The binding of ^{125}I-anti-H-2D antibodies (specific activity = 61%) to target (*d* x *k*) placentas is substantially higher than the binding of the same antibody to control (*d* x *d*) placentas on day 10 and 17 of gestation (Figure 12). Double reciprocal curves were plotted using the dose response values (Figure 13) and the intercepts of these curves on the Y-axis used to estimate the number of paternally-derived H-2D antigens on the allogeneic placenta. The density of paternal H-2D antigens on the allogeneic placenta is estimated to be in the order of 0.9×10^{12} determinants per gram and 1.9×10^{12} determinants per gram placenta on day 10 and on day 17 of pregnancy respectively. The density of paternal H-2K*k* antigens on the placenta is about 5 times higher than the density of H-2D*k* determinants.

F. Binding of F(ab)$_2$ Anti-H-2K by the Target Placenta

It is possible that the anti-paternal H-2 antibody binds directly to paternal H-2 determinants on placental cells exposed to maternal circulation; however, it is also possible that this antibody binds initially to Fc receptors on the placenta and is then transported to sites where paternal antigens are expressed. In order to verify whether the binding of this antibody initially takes place via its Fc portion, the binding of F(ab)$_2$ anti-H-2K was compared to the binding of intact anti-H-2K antibody to the allogeneic placenta. Identical binding patterns of both, the F(ab')$_2$ and the IgG antibody, would obviate the need for Fc mediated transport while a difference in the binding patterns would suggest that the anti-paternal antibody has to go through a process of Fc mediated transport in order to reach the antigenic sites in the placenta.

Dose response curves were constructed by determining the uptake, by the allogeneic and syngeneic placentas, of various doses of the radiolabeled F(ab')$_2$. Graded amounts (ranging from 5×10^4 cpm to 8×10^5 cpm) of the ^{125}I-F(ab')$_2$ antibody (specific

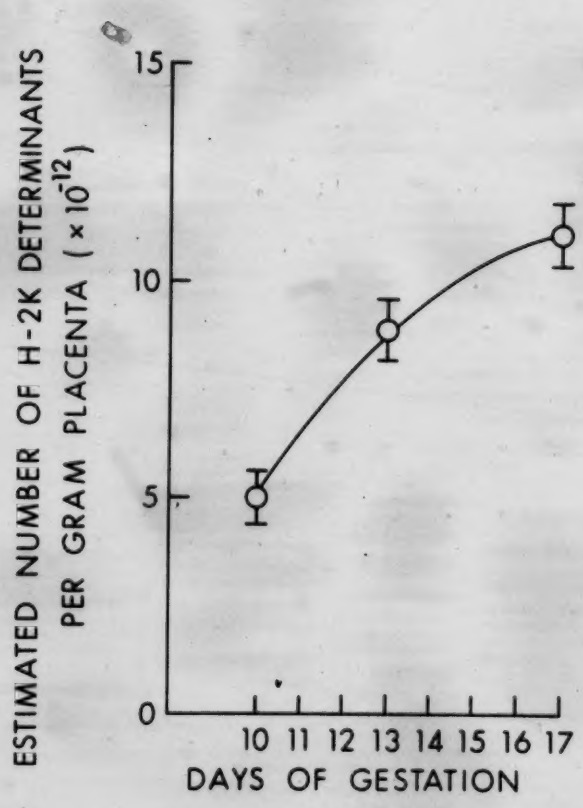


Figure 11 – The estimated density of paternal H–2K antigens on the placenta at different stages of gestation. The numbers of H–2K determinants per gram (±S.E.) are estimates for day 10, day 13 and day 17 placenta.

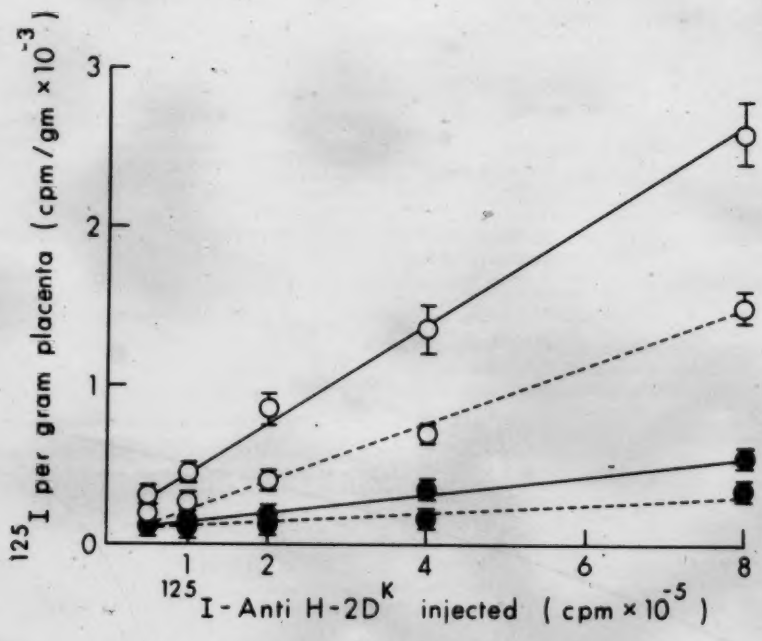


Figure 12 – Binding of radiolabeled anti–H–2D*k* antibody to target and control placentas on days 10 and 17 of gestation. (O——O target day 10 placenta; ●——● control day 10 placenta; O- - - -O target day 17 placenta; ●- - - -● control day 17 placenta)

activity=60%) were injected into syngeneically and allogeneically pregnant mice. The uptake of radiolabeled $F(ab')_2$ by the placentas was then assessed 6 hours later. The dose response curve for $F(ab')_2$ placental uptake is essentially identical to that obtained using intact antibody (Figure 13A). These data were replotted in double reciprocal fashion to determine the number of H-2K antigens detected by the $F(ab')_2$ preparation (Figure 13B). This estimate is 10.4×10^{12} ($\pm 0.83 \times 10^{12}$) determinants per gram *d* x *k* placenta, which is statistically similar to the estimate of 11.1×10^{12} per gram obtained using the whole immunoglobulin.

It is clear therefore, that Fc receptor binding is not a necessary pre-requisite for H-2K binding from the maternal side.

G. Effect of Fetectomy on Placental Immunoabsorption of Anti-Paternal H-2K Antibody

It has been suggested that the immunoabsorbent ability of the placenta may be attributed to the presence of fetal lymphoid cells expressing antigens of the paternal haplotype, circulating in the placenta (Swinburne, 1970). On the other hand, the placenta might be capable of synthesizing its own H-2 antigens and in that case the immunoabsorbent effect would not be dependent on fetal circulation through the placenta. In order to determine whether the immunoabsorbent capacity of the placenta can be influenced by the lack of fetal cells in the placenta, pregnant mice were fetectomized on day 11 of gestation and the binding capacity of the placenta then measured at different intervals following fetectomy. Non-fetectomized placentas in the opposite horn and non-fetectomized mice served as controls.

When only one uterine horn was fetectomized, the fetuses in the other horn usually developed normally and were delivered in a normal manner. The fetectomized placentas developed slowly as compared to the controls and the increase in weight of the fetectomized placentas during gestation was minimal (Table 2).

Mice were fetectomized on day 11 and the uptake of anti-paternal antibody measured 2, 4 and 6 days after fetectomy. 2×10^5 cpm of the radiolabeled anti-H-2K antibody were injected into each fetectomized or control mouse and the binding of antibody assessed 6 hours later. The uptake of antibody per gram placenta did not

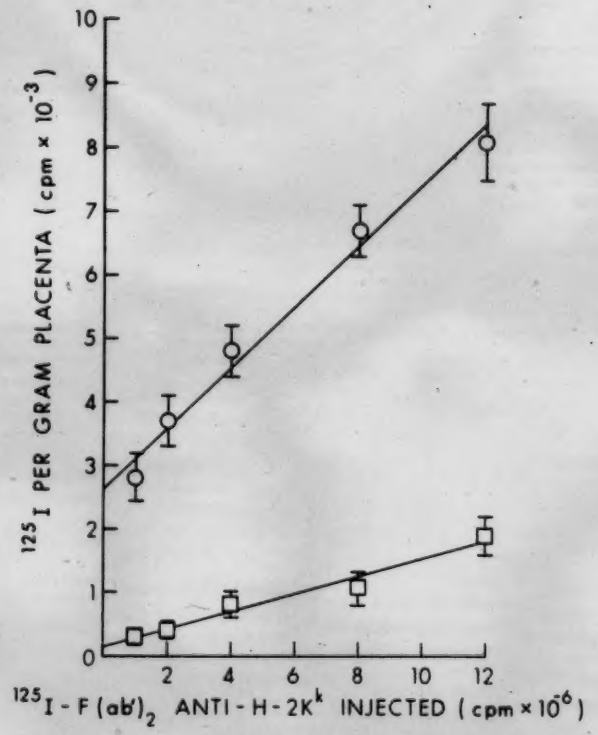


Figure 13A – Binding of F(ab)$_2$ anti–H–2K*k* to target *d*x*k* (○) and control *d*x*d* (□) placentas on day 17 of gestation.

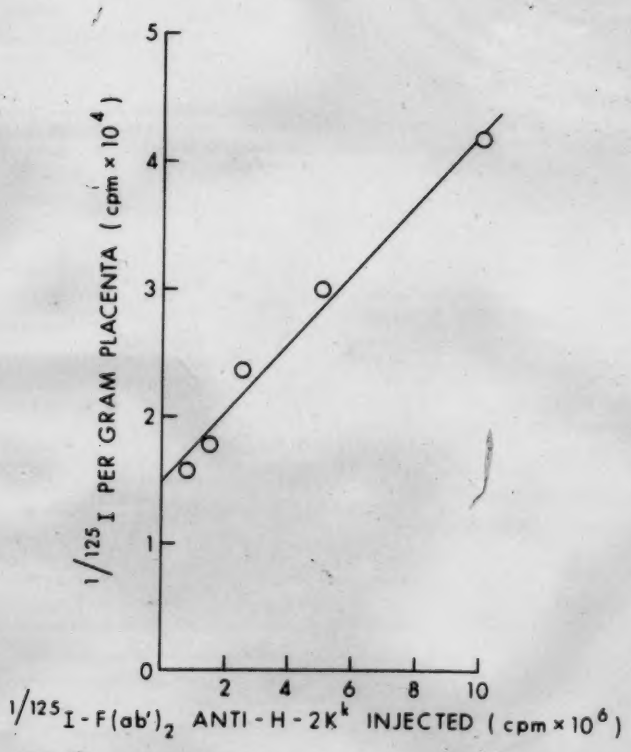


Figure 13B – Determination of placental immunoabsorbent capacity for F(ab')$_2$ anti–paternal H–2K*k* antibody. The intercept of the double reciprocal dose response curve was used to calculate the placental capacity for F(ab)$_2$ anti–paternal antibody.

TABLE 2
Weight in grams of placentas obtained from fetectomized and non-fetectomized uterine horns.*

PLACENTAS FROM	NUMBER OF DAYS POST-FETECTOMY 2	4	6
Fetectomized horns	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.02
Control horns	0.12 ± 0.01	0.17 ± 0.02	0.18 ± 0.02

* Fetectomy was performed at day 11 of gestation.

change when examined 2, 4 and 6 days post-fetectomy (Table 3). This indicates that the placenta is capable of synthesizing its own H-2 antigens after day 11, suggesting that a regular influx of fetal cells to the placenta is not necessary for the placenta to be able to absorb anti-fetal antibodies from maternal blood.

H. Autoradiographic Localization of Paternally-Derived H-2 Antigens in the Placenta

These studies were performed with the objective of localizing paternal H-2 antigen-bearing cells in the allogeneic placenta. Three different antibody preparations were used in this study : ^{125}I-anti-H-2K*k*. ^{125}I-F(ab')$_2$ anti-H-2K*k* and intrinsically labeled ^{3}H-anti-H-2K*k*. Using the *in vivo* procedure described in the MATERIALS AND METHODS section, the binding pattern of anti-paternal H-2K antibodies to target allogeneic placentas and non-target syngeneic placentas was examined.

The control placentas showed no labeling whatsoever, with all the three antibody preparations tested. The perfusion step apparently removed unabsorbed antibodies from the vasculature of the placenta.

In the target placentas, binding was principally seen in two areas. The most intense binding was observed on the parietal endoderm cells in the lateral aspects of the placenta, where the yolk sac inserts into the lateral edges of the placenta (Figure 14). Some of these densely labeled cells clearly resemble macrophages or monocytes, are esterase positive and lie within the endodermal sinuses (the Crypts of Duval) (Figure 15, 16). Labeled monocytic cells were also observed in the allantoic mesoderm; some of these labeled cells appeared to be infiltrating the visceral wall of the endodermal sinuses suggesting that they are migratory cells.

The second major area of radiolabeling occurs in the spongiotrophoblast area. Many of the labeled cells lie in direct contact with maternal circulation as they border maternal blood spaces. The distribution of label in the spongy zone was uneven, with many areas appearing unlabeled, others exhibiting light to moderate labeling. The intensity of label per cell in this region was considerably lower than that seen in the endodermal sinuses, indicating that the paternal antigen density in this area is probably low (Figure 17, 18).

TABLE 3
Effect of fetectomy on placental immunoabsorption of anti-paternal antibody.*

CPM/GM PLACENTA OF FETUSES FROM	NUMBER OF DAYS POST-FETECTOMY 2	4	6
Fetectomized horns	1892 ± 212**	2391 ± 193	2885 ± 127
Non-fetectomized horns	2020 ± 69	2423 ± 49	2624 ± 171
Control mice (Non-fetectomized)	1884 ± 79	2596 ± 200	2924 ± 147

* Fetectomies were performed on day 11 of gestation.
** Each value represents cpm ^{125}I-anti-H-2K*k* per gram placenta ± S.D.

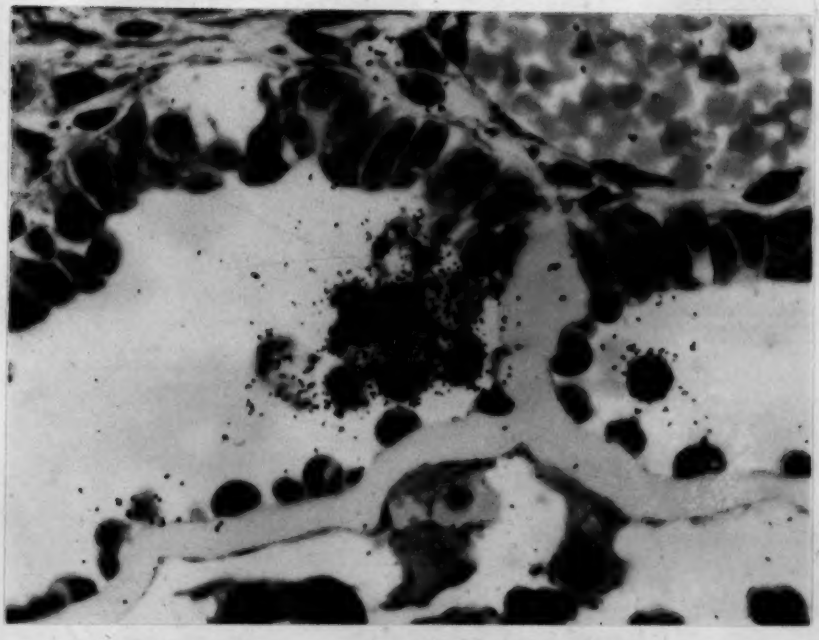


Figure 14 – Radiolabeled parietal endodermal cells within the endodermal sinuses of an allogeneic placenta.

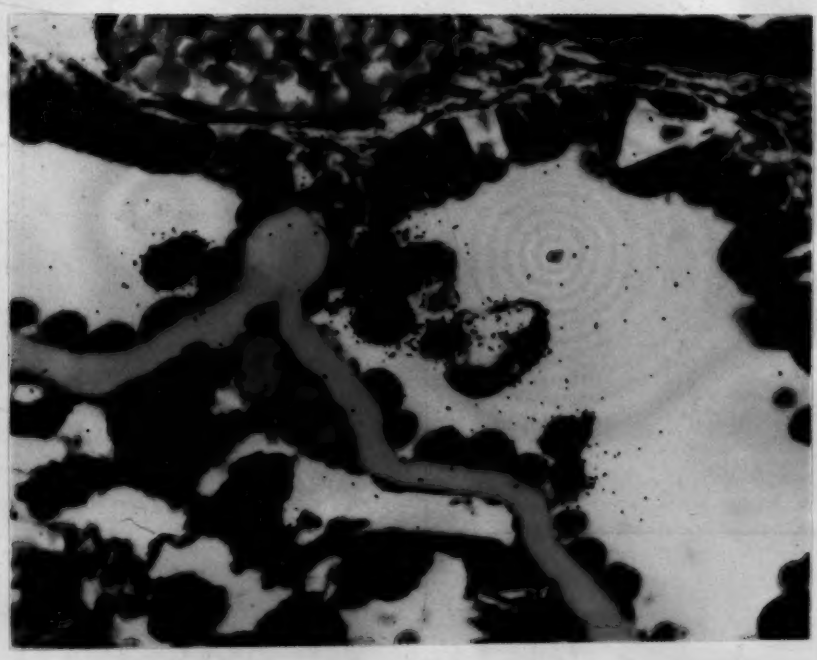


Figure 15 – Radiolabeled degenerating cells in the endodermal sinuses (x1140).

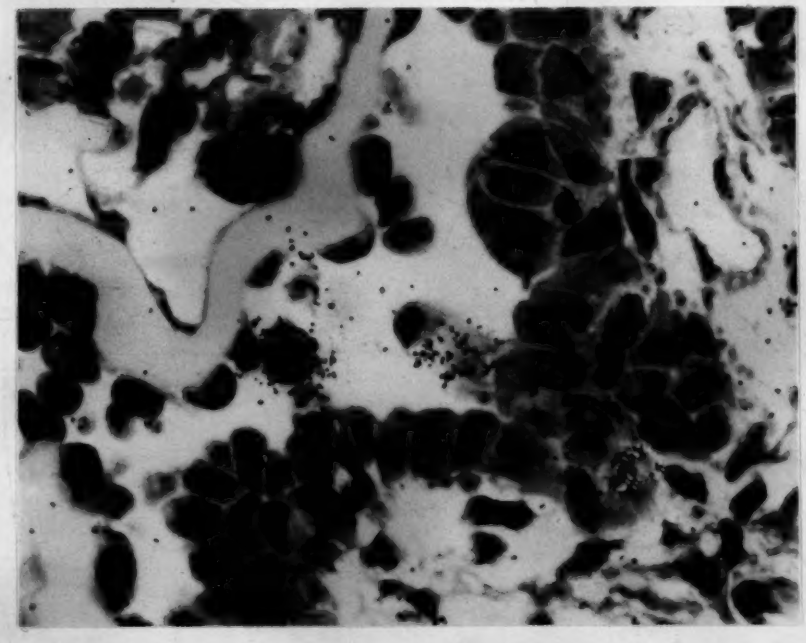


Figure 16 – Autoradiogram revealing densely labeled cells of monocytic or macrophage–like appearance in a target placenta. Labeled monocytic cells were found throughout the endodermal sinus region bordering the amniotic cavity on the fetal side of the placenta (x980).

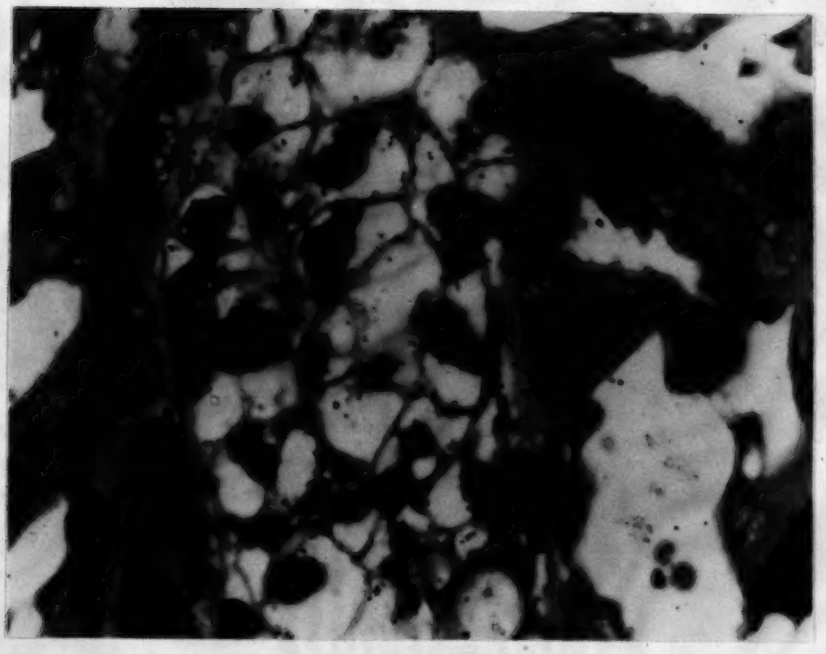


Figure 17 – Autoradiogram revealing labeled spongiotrophoblast cells in the spongy zone of a *dxk* placenta (x1140).

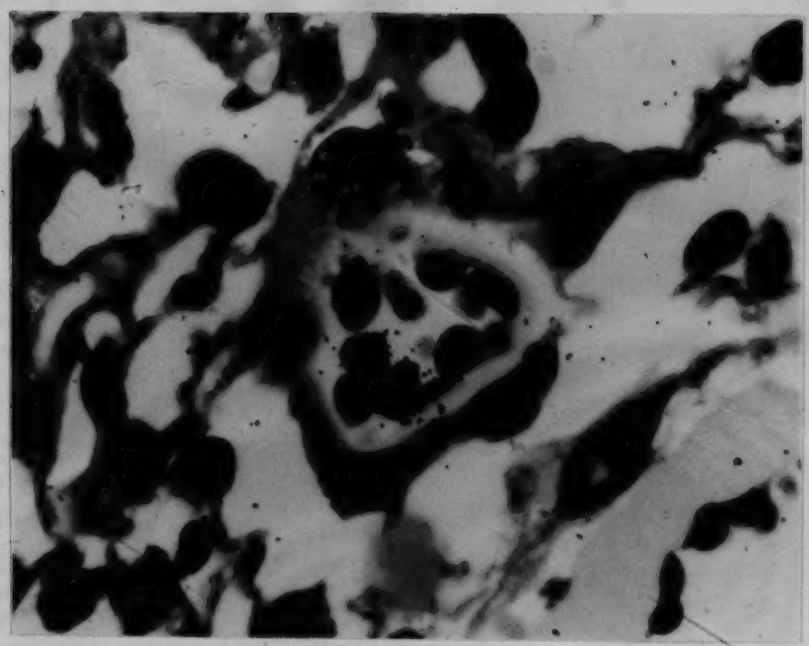


Figure 18 – Autoradiogram revealing labeled spongiotrophoblast cells in a target placenta.

Most of the labyrinthine region of the allogeneic placentas was devoid of label, except for an occasional lightly labeled cell. These uncommon cells were vacuolated suggesting that these may have been infiltrating spongiotrophoblast cells. Giant cells and decidual cells exhibit no anti-paternal H-2 antibody binding.

These patterns of labeling were seen at various time points after the injection of the antibody - from 1 hour to 8 hours - with very little qualitative differences in the distribution of the label. This pattern was also seen on day 13, day 15 and day 17 placentas. ^{125}I-F(ab')$_2$ binding was identical to that of the intact anti-H-2K antibody, confirming the previous finding that Fc binding plays no role in the binding of anti-paternal H-2 antibodies by paternal antigen bearing cells in the placenta.

The unique finding in these experiments was that paternally-derived H-2K, a major transplantation antigen, is present on spongiotrophoblast cells in direct contact with maternal circulation and on endodermal cells of the yolk sac plexus.

I. Antibody Turnover in the Placenta

In order to qualify as an efficient immunoabsorbent sponge, the placenta should be able to re-express it's antigens after antibody binding has occured; this may be accomplished by turning over the bound antibody either by digestion or by release as antigen-antibody complexes. This issue was addressed by determining the time required by the placenta to re-express its paternal H-2 antigens, after these antigenic sites are bound by antibodies injected into maternal circulation. To investigate the time kinetics of placental antibody turnover, unlabeled anti-H-2K*k* antibody was first injected at different time points prior to the estimation of the immunoabsorptive capacity of the placenta on day 17, using radiolabeled anti-H-2K*k* antibody. It was thus possible to determine the time taken by the placenta to regenerate its immunoabsorptive capacity i.e., it's H-2K antigens, after binding the unlabeled antibody.

The 75% inhibiting dose of the unlabeled antibody was first determined. In order to do this, 2 x 10^5 cpm of the ^{125}I-labeled antibody were mixed with graded amounts of unlabeled antibody and injected into BALB/cCR females pregnant by BALB/cCR or by C3H/HeJ males on day 17 of gestation. Six hours later, the placentas were removed and the residual radioactivity in the placentas determined. The inhibition of binding of antibody

to the target placentas (cpm of antibody bound to target placentas minus cpm of antibody bound to control placentas) was plotted as a function of the dose of unlabeled antibody (Figure 19A); this curve indicates the amount of unlabeled antibody required to inhibit the binding of 2 x 10^5 cpm of the labeled antibody by 75%. 50 *u*ls of the unlabeled antibody was required in order to cause 75% inhibition of the binding of 2 x 10^5 cpm of the radiolabeled antibody.

Therefore 50 uls of the ascites fluid were injected i.v. into target (*d* x*k*) and control (d x *d*)pregnant mice. At different time intervals after this (ranging from 2 hours to 72 hours), 2 x 10^5 cpm of the radiolabeled antibody were injected and the placentas harvested 6 hours later. The immunoabsorptive capacity of the placenta was plotted as a function of time (Figure 19B) and this curve indicates that the absorptive capacity of the placenta returned to normal 48 hours after the injection of unlabeled antibody. Thus paternally-derived H-2K antigens in the placentas are fully re-expressed 48 hours after binding the corresponding anti-paternal antibodies and are once again available for binding by anti-H-2K antibody.

J. Fate of Anti-H-2 Antibody in Target and Control Placentas

Cytoplasmic extracts prepared from target antigen-bearing placentas at different time points after the injection of 10^6 cpm of ^{125}I-labeled anti-H-2K*k* antibodies, (specific activity = 63%) were analyzed by S-200 chromatography. The elution profiles of these extracts indicated that a proportion of the radiolabel within placental cells was associated with fragments smaller than intact IgG. One hour after the injection of antibody the radiolabeled material eluted as three peaks, the first peak corresponding to undegraded IgG and the other smaller peaks corresponding to smaller fragments of the IgG protein (Figure 20A). Four hours after injection, the antibody appeared to have been degraded into even smaller fragments; indicating extensive degradation (Figure 21 A).

The control placentas presumably bound the antibody only by its Fc portion; no degradation of the antibody was evident until 6 hours after injection (Figure 20B, 21 B). When control placentas were examined 6 hours after antibody injection, some degradation of the labeled protein was observed (Figure 22 B).

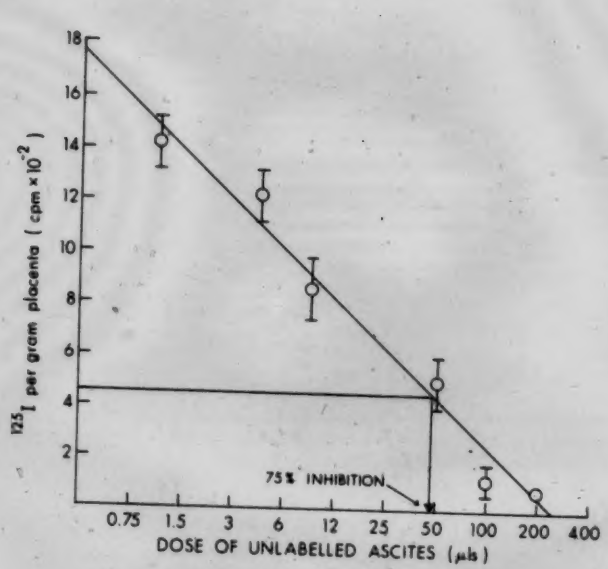


Figure 19A – Determination of the dose of unlabeled antibody required to inhibit the *in vivo* binding of ^{125}I–anti–H–2K to the placenta. Varying amounts of the unlabeled anti–H–2K*k* antibody were mixed with 2 x 10^5 cpm of the radiolabeled antibody and injected into target and control pregnant mice. Six hours later the placentas were processed and the cpm/gm assessed. Control *d*x*d* values were subtracted from the target *d*x*k* values before plotting as a function of the dose of unlabeled antibody initially injected.

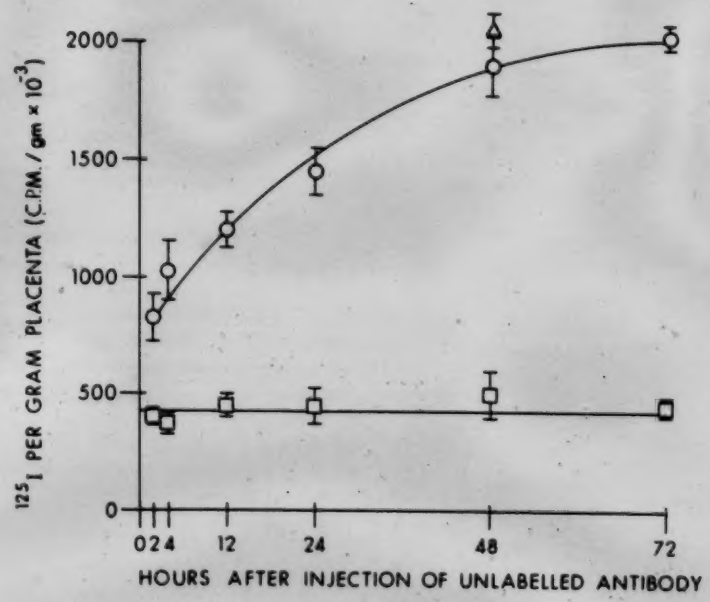


Figure 19B – Determination of the time required for the placenta to regain its immunoabsorbent capacity for anti–H–2K antibody. (□ syngeneic control; △ allogeneic control without unlabeled antibody; ○ allogeneic, with unlabeled antibody)

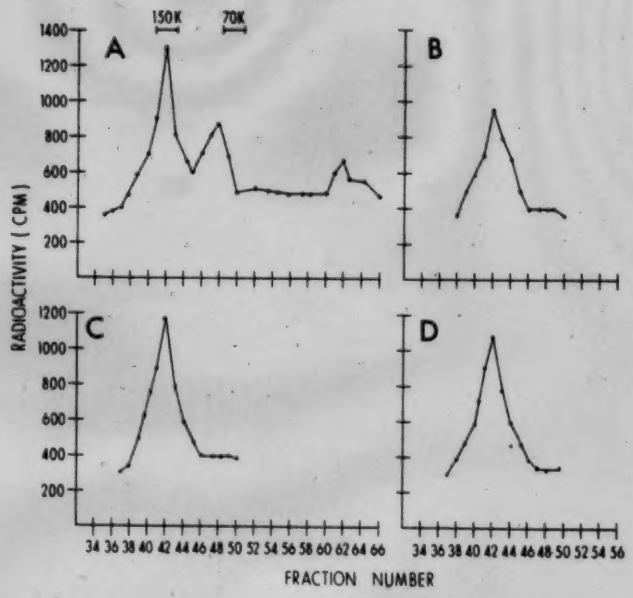


Figure 20 – Fate of anti-H-2K*k* antibodies 1 hour after injection *in* (A) target *d*x*k* placentas, (B) control *d*x*d* placentas, (C) serum from *d*x*k* pregnant females and (D) serum from *d*x*k* pregnant females.

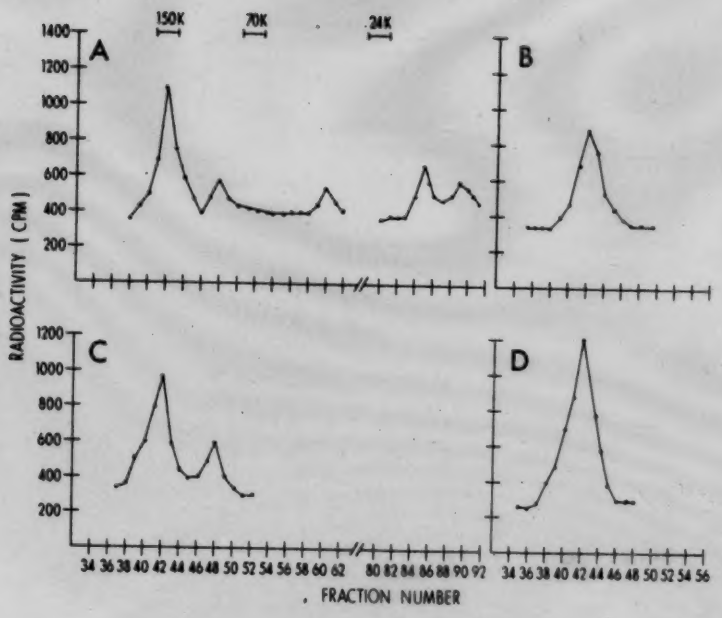


Figure 21 – Fate of anti-H-2K*k* antibodies 4 hours after injection *in* (A) target placentas, (B) control placentas, (C) serum from target pregnant females and (D) serum from control pregnant females.

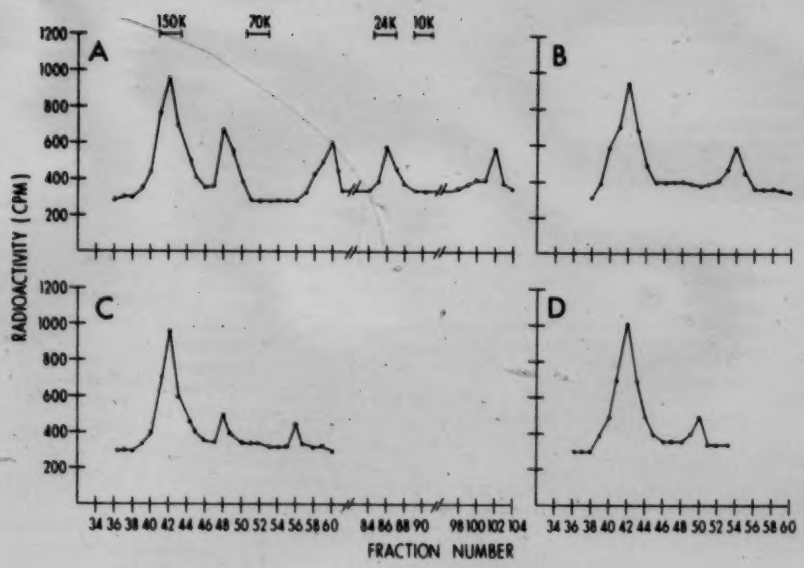


Figure 22 – Fate of anti-H-2K*k* antibodies 6 hours after injection *in* (A) target placentas, (B) control placentas, (C) serum from target pregnant females and (D) serum from control pregnant females.

There is therefore a marked difference in the manner in which anti-H-2 antibody is processed by target antigen-bearing and (control) placentas. Whereas the anti-H-2 antibody is ingested and degraded by the allogeneic placenta as early as one hour after injection; degradation by non-antigen bearing placentas appears to start six hours after injection.

Analyses of Serum for IgG Degradation Products

Sera were obtained from *d* x *d* and *d* x *k* pregnant females injected with ^{125}I-anti-H-2*k* and were analyzed for degradation products of IgG by S-200 chromatography.

Elution profiles of serum samples from target pregnancies indicate that the radiolabeled protein elutes in the position of intact IgG for the first two hours after the injection of antibody (Figure 20 C). 3 hours after injection, an additional peak is observed, indicating the presence of breakdown products of IgG in the serum (Figure 21 C). At the 6 hour point further degradation is observed (Figure 22 C).

Serum samples from syngeneically pregnant (control) females do not have demonstrable IgG breakdown products until 6 hours after the injection of antibody (Figures 20 D, 21 D, 22 D).

Though it is possible that the IgG degradation products seen in the serum may have been released by other tissues such as maternal liver or kidneys, the fact that these fragments appear in the serum of target pregnancies earlier than in the serum of control pregnant animals. suggests that the origin of the breakdown products seen in the serum is in the conceptus.

It is interesting to note that there was no evidence for the presence of antigen-antibody complexes in the serum of pregnant mice injected with anti-H-2K antibody. The elution profiles show that the labeled material, in no case eluted before IgG, indicating that within the limits of detectability, the anti-H-2K antibody is not released by the placenta into maternal circulation in the form of antigen-antibody complexes.

Anti-H-2 Antibody Degradation in the Liver

To compare the processing of anti-H-2 antibody by the placenta with that by the liver, BALB and (BALB xC3H)F_1 animals were injected with 10^6 cpm of ^{125}I-anti-H-2K*k* antibody and the livers excised at different time points thereafter. Cytoplasmic fractions were obtained from liver tissue (as in the case of placentas) and these fractions were examined for antibody breakdown.

The (*d* x *k*)F_1 livers showed no evidence of antibody degradation until 3 hours post-injection (Figure 23 A). IgG breakdown in the liver is first seen 3 hours after the injection of antibody (Figure 23 C). The non antigen-bearing control liver shows no evidence for antibody breakdown during until 6 hours after antibody injection (Figures 23 B, D)

This clearly indicates a difference in the time kinetics of antibody breakdown between the placentas and the liver. Whereas antibody breakdown in the placenta is seen as early as 1 hour after antibody injection, degradation in target livers appears to take place 2 hours later.

K. Examination of Paternal I-A Antigens on the Allogeneic Placenta

Class I (H-2K and H-2D) antigens constitute the first signal in the generation of specific alloreactivity in histoincompatible allograft reactions The second signal is supposed to be provided by Class II (Ia) antigens. As Ia antigens are potent helper determinants in allogeneic reactions, the presence of paternally-derived Ia antigens on the allogeneic placenta was examined using monoclonal anti-I-A*k* antibodies. ^{125}I-anti-I-A*k* antibody (specific activity = 66%) was injected into *d* x *d* and *d* x *k* pregnant females (4 x 10^5 cpm per animal) on day 17 of gestation and the uptake of radiolabeled antibody measured 6 hours later.

There were no detectable differences between control (*d* x *d*) and target (*d* x*k*) placentas, in the uptake of anti-I-A*k* antibody (Table 4).These results suggest that paternal strain I-A antigens (specificity 17) are not expressed in detectable levels on the placenta.

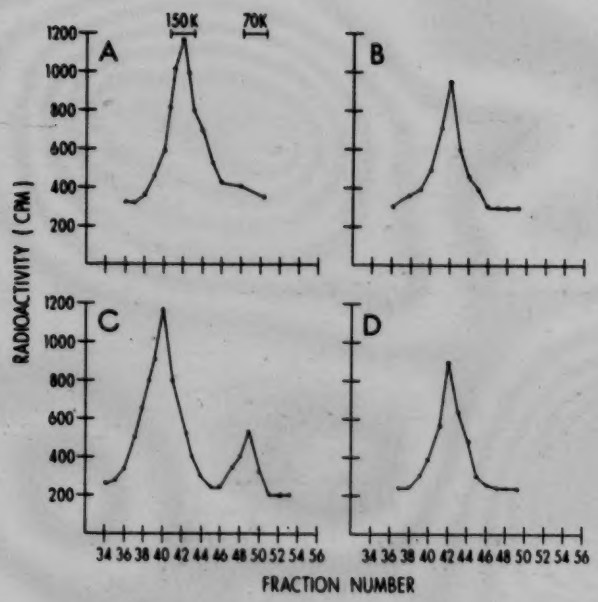


Figure 23 – Fate of anti-H-2K*k* antibodies in the (*d*x*k*)F_1 liver 1 hour (A) and 3 hours (C) after injection and in the H-2*d* (control) liver 1 hour (B) and 3 hours (D) after injection.

TABLE 4 Lack of expression of paternal I-A antigens by the allogeneic placenta.

DAY OF GESTATION	PREGNANCY	PLACENTAL CPM/GM ± S.D.	FETAL CPM/GM ± S.D.
13	Syngeneic (d x d)	1317 ± 247	633 ± 73
13	Allogeneic (d x k)	1198 ± 231	754 ± 81
17	Syngeneic (d x d)	1414 ± 105	1394 ± 149
17	Allogeneic (d x k)	1198 ± 106	1505 ± 301

IV. DISCUSSION

Pregnancy calls for some precisely coordinated physiological processes, among which are those that are required for protecting the embryo from maternal immune rejection. Many different mechanisms may contribute to this task – suppression of maternal lymphocytes, physical protection by decidual cells creating an immunologically privileged site for the embryo and perhaps most important, unique immunological properties of the placenta which may help avoid the potential rejection reactions resulting from fetal–maternal histoincompatibility.

There is no question that the trophoblast is a unique and peculiar tissue, especially from the perspective of the transplantation biologist. The trophoblast is in effect an intra–uterine allograft since it is a foreign tissue in direct and continuous contact with maternal tissues, either in close cellular abutment or bathed in maternal blood. Thus the trophoblast is in a strategic place where maternal–fetal interactions occur. While trophoblasts of ungulate species and carnivores generally invade the maternal uterus only slightly or moderately, the trophoblast of the hemochorial placentas of primates and most rodents, invades deep into uterine stromal tissues and opens up maternal blood capillaries to bathe the trophoblast surface. In the light of the anatomical arrangement of the placenta, one of the most crucial questions in maternal–fetal immunology relates to the antigenicity of the placenta. The transplantation of a tissue graft to a histo–incompatible host results in conventional host versus graft immunity leading to graft rejection. While a great deal is known about the major histocompatibility alloantigens on conventional tissue grafts, there has been a lack of conclusive data concerning the presence of histocompatibility antigens on the placenta.

The elegant studies of Rossant and her colleagues (1982) have provided convincing evidence regarding the role of the trophoblast in the immune protection of the fetus. Mus caroli blastocysts transplanted into the uterus of a Mus musculus female were rejected, apparently immunologically, by day 11 of gestation. However, blastocysts reconstituted by microsurgery, possessing ICM of the M. caroli type alone and the trophectoderm entirely of the M. musculus type, were viable and were delivered normally with no signs of immunologically mediated pathology. Furthermore, Croy *et al* (1982) transplanted EPC from M. caroli and M. musculus to kidney capsules of immunized M.

musculus mice and they observed a massive lymphocytic infiltration of M. caroli grafts alone, indicating that the xenogeneic trophoblast was recognized immunologically. The trophoblast thus seems to play an immunoprotective role. One other conclusion more pertinent to the present discussion, is that the antigenicity of the trophoblast appears to be crucial in determining the outcome of pregnancy. The presence of an intervening trophoblast expressing antigens foreign to the mother, appears to elicit anti-fetal reactivity in the mother, while a trophoblast not foreign to the mother protects the fetus that *is* foreign to her. One does have to take into consideration the fact that the pregnancies in these studies were interspecific and this thesis relates to intraspecific pregnancies. However, if these findings may be extrapolated to intra-specific pregnancies, then one may conclude that the trophoblast provides the major immunogenic stimulus to the maternal immune system. Lawler *et al* (1974) have demonstrated the presence of anti-HLA antibodies in sera after molar pregnancies which suggest that the mother need not be exposed to allogeneic fetal cells in order to mount a response against fetal antigens. The trafficking of immunogenic doses of fetal cells into the mother, has not been convincingly demonstrated. If the trophoblast is indeed the major immunogenic stimulus, then the antigenicity of the placenta, and of the trophoblast in particular, is very important. Considerable research has been directed at verifying whether paternally-derived MHC antigens are expressed by placental cells in allogeneic pregnancies. As discussed earlier (see INTRODUCTION) paternal H-2 antigens appear to be restricted to the embryonic compartment of the 7.5 day conceptus (Patthey and Edidin, 1975) and there may be a low density transient expression of H-2 antigens on the trophectoderm just prior to implantation (Jenkinson, 1981). At implantation, the embryo appears to present an outer surface deficient in alloantigens and consequently the possibilities of maternal recognition or immunological attack are reduced. Early during post-implantation development, the trophoblast of the EPC appears to be devoid of detectable H-2 antigens, but the ICM-derived egg cylinder continues to express H-2 antigens (Jenkinson and Billington, 1974). A variety of different *in vitro* tests have been employed to investigate the possible existence of H-2 antigens on the placenta. These include immunofluorescence, cytotoxicity, immune adherence and *in vivo* transplantation. Most of these approaches have failed to fulfil all the essential criteria for the unequivocal

demonstration of histocompatibility antigens on the cell surface of mature trophoblast cells. The drawbacks include lack of sensitivity and specificity and the use of morphological criteria alone to identify trophoblast cells in suspension is not entirely reliable. Furthermore, the question of whether these antigens, if present, are exposed to maternal circulation was unanswered. This question is especially important because the presence of paternal alloantigens in the placenta would be irrelevant if these antigens were to be sequestered from maternal circulation. This question was addressed in this study and has been resolved. The use of the *in vivo* approach, wherein radiolabeled anti-H-2 antibodies are injected into maternal circulation has provided us with the means to demonstrate that paternally-derived H-2 antigens are present in the placenta and that these antigens are accessible to maternal blood (Raghupathy *et al*, 1981).

When ^{125}I-labeled anti-H-2K*k* or anti-H-2D*k* antibodies are injected into pregnant females, a highly significant differential absorption of the label to the target (*d* x *k*) placentas as opposed to the control (*d* x *d*) placentas was observed. Two specificity controls were included in studies previously reported by Wegmann *et al* (1979) to rule out non-specific effects. ^{125}I-labeled non-specific IgG did not show the differential placental uptake. Syngeneic and third party controls appeared identical in their labeling pattern, if the third party strain was noncross-reactive with the target strain. If the third party strain was cross-reactive, an intermediate amount of labeling was obtained. These controls rule out differential binding on any other basis than H-2 (Wegmann *et al*, 1979) and the simplest interpretation of these results is that paternally-inherited H-2 antigens are present in the placenta in a manner accessible to maternal blood. The use of monoclonal anti-H-2K and anti-H-2D antibodies has helped improve the the genetic definition of the immunoabsorptive antigens.

That the antibody was not artifically altered during its preparation has also been previously demonstrated (Wegmann *et al*, 1980). Unlabeled antibody was tested for its ability to competitively inhibit the binding of purified ^{125}I-labeled antibody to H-2*k* leukemia cells *in vitro* and to the placenta *in vivo*. This study demonstrated that the labeled antibody preparation and the unlabeled antibody were both competing for the same binding sites on the leukemia target cells. The purified and labeled antibody is therefore unaltered with respect to its combining site.

Purified, radiolabeled antibodies, with specific activities of at least 60% were used in this study to estimate the number of placental paternally-derived H-2K and H-2D antigens, functionally exposed to the maternal circulation. The use of double reciprocal curves plotted from dose response curves permits the estimation of target antigens on tissues *in vivo* in a simple and straightforward manner. The earliest gestational stage the placentas were investigated for paternal H-2 antigens, was day 10 and at this stage H-2 antigens are clearly detectable. The densities of H-2K and H-2D antigens on the placenta continue to increase until day 17 of gestation. Thus the absorptive capacity of the placenta increases throughout gestation. The actual densities of H-2 antigens measured is probably a minimal estimate. First, the estimates obtained assume that the mother makes no antibody response, i.e., that *all* available antigenic sites are exposed. Second, the assumption is made that there is no antibody turnover in the placenta, which, based on evidence described in this thesis, is incorrect. Time course experiments indicated that maximal antibody binding to the placenta occurs 6 hours after the injection of antibody. Therefore all quantitation studies were performed using this parameter and during this six hour interval the antibody may have been turned over in the placenta; the binding of antibodies by tissue antigens is presumably a dynamic and continuous process. Although the actual estimates obtained may be low, the relative immunoabsorbent capacity measured shows a definite increasing trend from 5×10^{12} H-2K antigenic determinants per gram placenta on day 10 to 11×10^{12} determinants on day 17 and from 0.9×10^{12} H-2D determinants on day 10 to 1.9×10^{12} determinants on day 17.

Paternally-derived H-2K antigens are expressed at a density that is five times higher than the density of H-2D antigens; this difference in the densities is substantially significant. Chatterjee-Hasrouni and Lala (1979) found a somewhat lower binding of anti-H-2D antibodies as compared to anti-H-2K antisera, to dispersed placental cells *in vitro*; they attributed this difference to differences in antibody affinity and antigen immunogenicity. However, H-2K*k* antigens on spleen cells appear to be expressed in concentrations substantially higher than are H-2D*k* antigens (O'Neill and McKenzie, 1980). Furthermore, although H-2K and H-2D antigens appear to have equal effects on graft survival, the K and D loci display a certain asymmetry (Klein, 1975). H-2D determinants appear to be generally weaker than H-2K, as measured by MLR, g-v-h and other assays

(for review see Klein, 1975). Thus the asymmetry of K and D antigen densities in the placenta, may simply reflect the asymmetry observed elsewhere.

The experiments with $F(ab)_2$ anti-H-2K*k* monoclonal antibody lend credence to previous findings in this laboratory (Wegmann *et al*, 1979) that Fc receptor binding is not a necessary pre-requisite for placental uptake of anti-H-2K*k*. It is conceivable that while paternal H-2 antigens are accessible to maternal blood they are partially sequestered in that the anti-H-2 antibodies may have to go through a process of Fc binding and Fc-mediated transport to internal sites where H-2 antigens may be expressed. Radiolabeled $F(ab')_2$ anti-H-2K*k* was used to re-examine the immunoabsorbent capacity of the placenta. Dose response curves of the $F(ab)_2$ and intact IgG were both essentially identical. The estimated density of H-2K antigens on day 17 placentas was also essentially the same, when estimated using $F(ab)_2$ and the intact antibody. These results indicate that the $F(ab')_2$ fragments of the antibody have access to the antigens and the antibody therefore does not have to undergo initial Fc binding. This indicates that paternal H-2 antigens are exposed directly to maternal blood.

The survival and growth of the trophoblast in spite of its proximity to maternal tissues and its expression of paternal alloantigens, may be attributed to a low-density expression of these antigens. Furthermore, anti-H-2 antibodies may block these antigens on the placenta rendering them inaccessible to alloreactive maternal cells, if any.

In view of the expression of paternally-derived Class I MHC antigens by the allogeneic placenta, it was essential to ascertain whether paternally derived I-A antigens are expressed as well. The proliferation of specific alloreactive T cells is the first of a series of immunologic reactions that occurs in a histoincompatible allograft rejection sequence. At least two main signals are supposed to be involved in the activation of alloreactive T cells against allografts (Lafferty and Cunningham, 1975; Bach *et al*, 1976). Signal 1 is provided by the ubiquitously occuring Class I alloantigens, which induce precursor alloreactive T cells to proliferate and signal 2 is the help provided by specific T helper (Th) cells; these Th cells are supposed to be induced by Class II alloantigens (Bach *et al*, 1976). Thus Class I and Class II antigens appear to play potentially different roles in the series of events that comprise an allograft reaction. Ia antigens are suggested to play an important role in the *initial* triggering of an immune response to allogeneic tissue

(Thorsby *et al*, 1978). Therefore whilst viewing the conceptus as an allograft, it was considered essential to determine whether Class II alloantigens are indeed present on the placenta at the maternal-fetal interface.

Using radiolabeled monoclonal anti-I-A*k* antibody, no evidence was obtained for differential binding of anti-I-A*k* antibody to the target *d* x *k* placenta. The uptake of anti-I-A antibody by target and control antibodies was measured on day 13 and day 17 and the results indicate that paternally-inherited I-A molecules are missing from the placenta at the stages tested. An important point to consider is the sensitivity of this assessment technique. One may argue that there may be a low level expression of Ia antigens, below the level of detectability of this assay. The bulk of the evidence argues against such a conclusion. The lack of I-A antigen expression correlates with the results of Chatterjee-Hasrouni and Lala (1981) who used a sensitive radioautographic technique, using highly specific antibodies directed at Ia antigens. No significant level of Ia antigens was demonstrable on the surface of trophoblast cells at gestational stages ranging between 12 and 17 days. The same dissociated cells did exhibit binding of anti-H-2D antibodies, demonstrating the reliability of the technique. Jenkinson and Searle (1979) have previously reported that post-implantation embryos, early trophoblastic tissues and trophoblasts from definitive placentas, were uniformly negative when tested in an immunoperoxidase assay with anti-Ia antiserum. The monoclonal antibody described in this thesis is directed at Ia specificity 17 and the antiserum used by Chatterjee-Hasrouni and Lala (1981) was capable of detecting Ia specificities 1, 2, 3, 7, 15, 17, 19 and 22. Thus a whole range of Ia specificities have been investigated and the lack of Ia antigens on the placenta has been confirmed by other laboratories too. The absence of Ia antigens on murine trophoblast cells corresponds with the reported absence of Ia-like antigens on the human placenta (Faulk *et al*, 1977).

In summary, there is a consensus that the placenta expresses paternally-inherited Class I alloantigens but lacks Class II antigens. A question that is highly relevant at this point is whether the lack of Class II antigens may be responsible for the absence of successful maternal anti-fetal rejection reactions in pregnancy.

Analyses of MLR and GVHR assays, which measure primarily the proliferative phase of the allograft reaction, indicate that the strongest proliferative stimulus is

provided by Class II antigens on stimulator cells; there seems to be no doubt that lymphocyte-activating determinants in MLR are carried by the same molecules that bear the serologically detectable Ia determinants (Klein, 1981). However, somewhat weaker and more variable stimuli are provided by Class I antigens as well (reviewed by Klein, 1976). It seems fairly clear that the recognition of Class I target determinants may not be a sufficient stimulus for the *complete* differentiation of T cells, and second signals are needed (Bach, 1980). This second signal appears to be provided by Class II antigens but an interesting fact is that the second signal may also be induced in the absence of "foreign" Class II antigens. After and Bach (1979) suggest that the alternative second signal in an MLR may be the product of a non-Tc cell activated by the same or the Class I alloantigens. Lafferty (1980) suggests that the second alternative signal is provided by a lymphocyte costimulator which is a product of a Th cell in the *stimulator* cell population. Thus, Ia alloantigens are not indispensable but they do provide the strongest primary induction in the initial phases of the reaction.

In actual allograft rejection assays, Class I antigenic differences alone can lead to rejection of skin allografts, and at least to some tumor allografts and to the rejection of at least some heart grafts (Klein 1977). In man, the survival of transplanted tissue in non-sensitized individuals is prolonged by identity for either Class I (HLA-A, -B, -C) or Class II (HLA-D) antigens but of the two types of MHC determinants, identity for Class II antigens has greater predictive significance for the duration of graft survival. Thus a matching for Ia antigens between donor and host appears to be more important than Class I matching (Thorsby *et al*, 1978).

Thus the role of Class II antigens in the rejection phenomenon has not been unequivocally resolved. The results of MLR and GVHR assays suggest that Class II differences are very potent in eliciting proliferative responses and Class I antigens are relatively weak in this regard (Klein and Park, 1974) but allograft rejection assays indicate that grafts differing at Class I loci are rejected rapidly. Thus an essential role for Class II antigens may be circumspect, but their role in maximizing this response cannot be ignored. Therefore one may attribute the lack of strong cell-mediated anti-fetal reactions during pregnancy to the absence of Class II determinants. A parallel to this may be seen in other tissues. Murine thyroid follicle cells and pancreatic epithelial cells

express modest levels of Class I antigens, but not Ia antigens and these paranchymal cells are the least immunogenic component of their respective tissues (see Head and Billingham, 1982). Organ allografts, when depleted of passenger lymphocytes (Billingham, 1971) by either *in vitro* culture or anti-lymphocyte serum treatment (Lafferty and Woolnough, 1977; Lacy *et al*, 1979), survive for longer periods of time, presumably due to the depletion of Ia-bearing lymphocytes. This hypothetical link between the lack of Class II antigens on the placenta and the lack of anti-fetal cell-mediated immunity, may not be tenous, especially if Class I antigens are expressed on the placenta in low concentrations in which case a lack of potent helper determinants may determine whether or not maternal immunocompetent cells are induced to proliferate. This configuration of alloantigens on the placenta may direct the maternal response to a humoral rather than a cellular type and furthermore may shift the balance of the humoral immune response in favor of an enhancing rather than a cytotoxic type. Antigen shed directly by the trophoblast or associated with trophoblastic deportation may be significant here. The exposure to alloantigenic material by the intravenous route favors the production of enhancing rather than cytotoxic antibodies. The mother is stimulated by what in effect is a chronic exposure primarily via the circulation, to perhaps low antigenic doses, and this may also result in the production of enhancing antibodies. Another contributor to this apparent immunodeviation seen in pregnancy could be suppressor cells in the fetal environment. Slapsys and Clarke (1982) and Clark *et al* (1979) have detected suppressor cells in the decidua and DLN of pregnant mice and these suppressor cells may also prevent the generation of cytotoxic cells directed at fetal antigens.

The ultimate cause or causes of this deviation of maternal immune responses is still unresolved but it is clear that cellular reactivities are absent and anti-fetal humoral immunity is often detectable. The maternal-fetal relationship was viewed as an allograft/host situation as early as in 1964 (Billingham) and several workers have attempted to draw parallels between the two situations. However, several significant differences exist between pregnancy and the classical allograft situation. The hallmarks of a host's response to major alloantigens on a graft include: hypertrophy of draining lymph nodes, production of cytotoxic antibodies, generation of sensitized T cells and the establishment of specific immunologic memory manifested as a heightened reactivity

against a similar second graft. The maternal immune response against her fetus is substantially different:

(i) hypertrophy of the draining lymph nodes, though it does occur in allogeneic pregnancies, is not a consistently demonstrable feature;

(ii) while cytotoxic antibodies are generated, the major alloantibody response in pregnancy appears to be of the enhancing type (Bell and Billington, 1981);

(iii) while the intrinsic reactivity of maternal lymphocytes is intact, T cells directly cytotoxic for paternal target cells are non-existent or very weak at the most; and,

(iv) specific immunologic memory against paternal grafts is not observed.

The survival of the fetus therefore may be primarily attributed to the absence of strong, cellular anti-fetal reactivity. However, the presence of cells sensitized to fetal antigens induced by experimental priming, does not compromise pregnancy (Heslop *et al*, 1954; Lanman *et al*, 1962; Wegmann *et al*, 1979). The placenta therefore must be capable of acting as a shield against maternal cells, either by non-specific anatomic means or by immunologic mechanisms.

The importance of the cell-mediated limb of the immune system allograft rejection reactions has become evident over the last two decades and there tends to be a generalization that antibodies mediate none of these allograft reactions. While this conclusion is true in skin graft reactions in mice, it does not always apply to all tissue grafts. Antibodies clearly mediate hyperacute renal allograft rejection in man and other species, they are often found in chronically rejecting grafts and they also cause the rejection of lymphoid grafts in mice (reviewed by Loveland and McKenzie, 1982). Under certain circumstances, antibodies alone can produce skin graft rejection in mice when an extraneous source of complement is provided (Winn *et al*, 1973). Obviously, antibodies elicited during pregnancy do not harm the fetus. In the experiments described in this thesis and previously by Wegmann *et al* (1977, 1979) cytotoxic antibodies having *in vitro* and *in vivo* capabilities were injected into maternal circulation; and fetuses were delivered with apparently normal characteristics. Furthermore, the anti-paternal H-2 antibodies used in these studies are of the IgG_2 subclass and are therefore capable of transplacental passage, so some form of size dependent sequesteration of these antibodies by the placenta fails to explain the lack of harm to the fetus. A conjecture that

has been reiterated several times in the past (Swinburne, 1970; Billington, 1976; Billington and Jenkinson, 1975) and for which sufficient evidence was not available until now, is that the placenta may serve as a specific antibody sponge by binding anti-fetal antibodies. The placenta would have to be a "specific" immunoabsorbent in excluding potentially deleterious antibodies, because the fetus, at least in mice and humans, depends on maternal transfer of antibodies essential for defending itself against infections. This type of protection would almost be necessary (Billington, 1976) if there were effective complement levels in the fetus, since it would appear that the density of histocompatibility antigens on most fetal tissues are sufficient for immune attack (Edidin, 1972). The results dsescribed in this thesis provide evidence for the idea that the placenta serves as a paternal strain antigen immunoabsorbent. The differential absorption of anti-H-2 antibodies by target and control placentas continues up to 72 hours after the injection of the antibody and the levels of antibody in target fetuses is consistently lower than that in the control fetuses up to 72 hours at least, after which the antibodies are presumably degraded anyway. Since one would expect an increasing maternal immune response to the fetus as pregnancy proceeds, it is interesting that the immunoabsorptive capacity of the placenta increases on a per gram basis, thus even more so for the intact placenta, because the placenta also increases in weight throughout gestation. In the past others have shown that where a fetus possesses an antigen to a maternal cytotoxic antibody, antibody activity can be detected in the placental eluate but not in the neonatal serum and conversely where the fetus lacks an antigen to maternal antibodies, antibody is not found in the eluate but is found in the neonatal serum. This does not, however, prove that the placenta is an immunoabsorbent, because one could argue that the absence of anti-paternal strain antibodies in the fetal circulation could simply indicate that they are absorbed elsewhere in the fetus. The current experiments therefore appear to be the first to clearly indicate a specific immunoabsorbent shielding role for the placenta (Raghupathy *et al*, 1981).

Similar shielding mechanisms may be operative in human pregnancy. Even if the human trophoblast is devoid of paternally-derived fetal antigens, stromal cells in the chorionic villi do express HLA antigens and anti-fetal antibodies may be absorbed by this cell layer. It has been suggested that on rare occasions HLA antibodies may overflow the

absorptive capacity of the placenta and cross into the fetal circulation, possibly causing fetal disorders such as neonatal thrombocytopenia (Jeannet *et al*, 1977). There is no evidence for this possibility as yet. It is also likely that the occurence of erythroblastosis fetalis caused by anti-Rh antibodies attacking a Rh positive fetus, may be attributed to a lack of Rh antigenic determinants on the placenta as a result of which anti-Rh antibodies would have unimpeded access into the fetus.

As the placenta is involved in absorbing anti-fetal antibodies from maternal blood, it is surprising that these antibodies do not mediate a substantial attack on the placenta itself. Billington (1979) suggests that some unique immunological properties of the trophoblast may contrive to avert the anticipated rejection reactions. Various mechanisms have been postulated to be effective in protecting the placenta from maternal antibodies. The placenta has been suggested to be covered by a sialomucoprotein layer which may impede the effector limb of the imune response arc (Taylor *et al*, 1979). This fibrinoid layer is not a universal occurence hence a protective role for this protein has yet to be firmly established. Blocking antibodies may protect the placenta from sensitized lymphoid cells of the mother (Rocklin *et al*, 1976). Other postulated mechanisms include low antigen density, rapid shedding of antigens and antigenic modulation by antibodies (Billington, 1979). and rapid, efficient repair processes to promote recovery (Jenkinson and Billington, 1974). The abovementioned mechanisms may ensure the survival of the fetus and of the placenta despite the presence of potentially detrimental maternal anti-fetal antibodies. Thus fetal survival would be ensured by the immunoabsorbent capabilities of the placental and placental escape from antibody mediated damage may be ensured by any of the immunological evasive tactics described above.

In summary, cells in the murine placenta express paternal histocompatibility antigens and thus serve as a specific filter barring the transplacental passage of harmful maternal antibodies to the fetus. Where do the immunoabsorbent cells in the placenta originate from? It has been suggested that fetal lymphoid cells could migrate to the placenta where they might provide the placenta with its immunoabsorptive capacity (Swinburne, 1970). On the other hand, the placenta may synthesize its own antigens *per se*. In order to determine whether the placental immunoabsorptive capacity for anti-fetal antibodies is indeed dependent on fetal circulation, we estimated the absorptive capacity

of the placentas after the fetuses had been removed on day 11 of gestation. The amount of antibody uptake per gram placenta did not change, when assessed 2, 4 and 6 days after fetectomy. This indicates that elements from fetal blood do not participate in the placental immunoabsorbent function. After fetectomy, the placenta was found not to increase substantially in size and the increase in weight of the placenta on day 17 is at most two-fold. However, the absorptive capacity per gram placenta, i.e., the density of paternal H-2K antigens per gram placenta undergoes its normal increase per gram, despite fetectomy. Hence, the placenta is capable of synthesizing its own paternally-derived H-2 antigens indicating that a regular influx of fetal lymphoid cells, from the fetus to the placenta is not required for the placenta's immunoabsorptive function, at least after day 11 of gestation. These studies do not rule out the possibility that fetal cells could have migrated to the placenta before day 11, where they could have proliferated to form a layer of cells absorbing anti-fetal H-2K antibodies from maternal circulation. These findings also provide some insight into the type of cell that may be involved in antibody binding.

Davies and Glasser (1965) performed extensive studies to observe the histological changes in the placenta as a result of fetectomy. The principal changes occuring 4 to 6 days post-fetectomy include, total degeneration of fetal mesenchyme, with the possible exception of cells in the endodermal sinuses, and a lack of increase of the labyrinthine trophoblast which accounted for minute portion of the total placenta at the end of gestation. However, the spongiotrophoblast area increased in area until at the time of parturition, it formed the bulk of the residual placental mass. These observations correlate very well with the idea that the spongiotrophoblast cells are involved in absorbing maternal anti-fetal antibodies. The results of the fetectomy studies indicate that the density of paternal H-2 antigens per gram fetectomized placenta increases in a manner identical to a normal placenta, suggesting that a substantial proportion of the labeling must be taking place in the spongy area, because fetectomy is accompanied by an involution of fetal mesenchyme and the labyrinthine regions accompanies fetectomy (Davies and Glasser, 1965).

These observations led us to address the question concerning the precise location of paternal antigen-bearing cells in the placenta. We used the *in vivo* model and

an autoradiographic technique to identify placental cells that bear paternal H-2 antigens. ^{125}I-labeled anti-H-2K*k* and antibody intrinsically labeled with ^{3}H-leucine used as tracer reagents were injected into the circulation of pregnant female mice and the binding of antibody examined by autoradiography at various intervals thereafter. This is a definite improvement over other *in vitro* approaches, because antigen-bearing cells that are physiologically relevant would be discernible by this *in vivo* technique. Control placentas showed no antibody binding whatsoever. Target placentas exhibited binding principally in two areas. The most intense binding was seen in the endodermal sinuses; many of the labeled cells appeared to be esterase positive macrophage-like cells and are probably of fetal origin as they are found on the fetal side of the Reichert's membrane. The second major area of antibody binding occured in the spongiotrophoblast region and the radiolabel was observed on trophoblast cells in direct contact with maternal circulation. The density of paternal antigens on spongiotrophoblast cells appeared to be considerably lower than that seen on cells in the endodermal sinuses, based on the density of the label per cell in these two areas. The labyrinthine trophoblast cells and the trophoblast giant cells showed no labeling with anti-paternal H-2 antibody. Thus there are at least two barriers to the passage of potentially harmful maternal antibodies in the mouse placenta: one in the spongy zone where maternal blood bathes spongiotrophoblast cells, and another, composed perhaps of specialized immunoprotective cells, in the endodermal sinuses, a region of antibody transport activity. By virtue of its H-2 antigen expression, the spongiotrophoblast could contribute to the immunoabsorptive capability of the placenta without compromising the transport processes of the placenta, because it lacks a fetal blood supply hence may not be directly involved (Jenkinson and Owen, 1980). The labyrinthine trophoblast region which consists of fetal blood vessels and mesenchyme covered by trophoblast, is devoid of paternal H-2 antigens. Similar results have been reported by Jenkinson and Owen (1980) who studied the expression of H-2 antigens on cultured cells taken from the spongy zone and from the labyrinthine region of the placenta. They found that cells selected from the spongy zone and not the labyrinthine trophoblast express paternal H-2 antigens.

Thus paternal antigens are expressed at the maternal-fetal interface on trophoblast cells, in contact with maternal decidual cells. Hence spongiotrophoblast cells

may influence maternal immune responses, by promoting the formation of enhancing antibodies. The paternal antigen-bearing macrophage-like cells in the endodermal sinuses may function as scavengers by interiorizing antigen-antibody complexes and may thus serve to eliminate antibodies absorbed by the placenta. Indirect immunofluorescence studies using specific antibodies have helped identify macrophages associated with murine placenta and yolk sac (Wood, 1980). Their location labels them as being of fetal origin and they may derive from primitive mesenchymal cells (Wood, 1980) and furthermore, may be similar to the wandering macrophage-like cells seen in the endodermal sinuses. These cells are interposed between the yolk sac cells and fetal blood channels, and may serve as immunoabsorbent cells as well. Thus different functions of the placenta may be localized in different anatomical regions (Jenkinson and Owen, 1980).

Chatterjee-Hasrouni and Lala (1982) have reported findings that differ substantially from those described in this thesis and those of Jenkinson and Owen (1980). They injected ^{125}I-labeled anti-H-2K antibodies into the placental branches of the uterine artery and obtained placentas 15 minutes later. Autoradiography studies apparently revealed that the labyrinthine trophoblast cells were labeled, while the cells in the spongy zone were not. These results signify a significant departure from our results. The differences in labeling may be reconciled by the fact that the placentas were exposed to antibody for a very short time, which may be inadequate for proper binding of antibody.

In a summary of the results discussed above, we may conclude that paternally derived Class I molecules are expressed on spongiotrophoblast cells and on some macrophage-like cells in the lateral aspects of the placenta, and that these antigens serve to bind anti-paternal antibodies from maternal circulation thereby preventing their access to the fetus.

We then addressed the question concerning the ability of the placenta to avoid saturation by maternal antibodies; pregnant females may produce anti-fetal antibodies throughout the latter stages of pregnancy and a continuous process of turnover and re-expression of paternal MHC antigenic determinants would be one way of preventing a complete saturation of all the determinants on the placenta, which could result in a leakage of antibodies into fetal circulation. The binding pattern of antibody over time

after a pulse of radiolabeled anti-H-2K antibody, indicated that there was an initial rise followed by a fall of antibody binding in the target placenta (Figure 5); this suggests that the antibody was being turned over, either by digestion *in situ* or by release from the placenta, perhaps as antigen-antibody complexes. We have tried to address this issue by determining whether paternal H-2K antigens in the placenta could be re-expressed after they are bound by antibodies. We first determined the dose of unlabeled anti-H-2K*k* monoclonal antibody which would cause 75% inhibition of the binding of radiolabeled antibody in the placenta, when measured six hours after injection. This was accomplished by injecting increasing amounts of unlabeled antibody along with a single dose of the radiolabeled antibody. The 75% inhibitory dose of unlabeled antibody was then added at various time points prior to the administration of a single dose of radiolabeled antibody, the latter always administered on day 17 of gestation. Using this approach, the turnover phenomenon was confirmed, and a maximal estimate was obtained for the rate of antibody turnover. We found that it took 48 hours to restore the placental immunoabsorptive capacity to normal after the addition of unlabeled antibody. This is a maximal estimate of the turnover period, because a give antigenic site on the placenta could have been replaced a number of times during those 48 hours. Thus while the *actual* turnover period for an antibody molecule bound to the placenta is expected to be less than 48 hours, the fact that the antigenic capacity of the placenta is re-expressed after having been bound by antibodies is beyond doubt.

This capability of the placenta to re-express its antigens is an essential criterion for it to serve as an efficient immunoabsorbent barrier. A logical question that follows the previous observation concerns the fate of the antibody; is the disappearance of the antibody from the placenta due to an *in situ* digestion process, or is the antibody released along with antigen as complexes, into the maternal circulation? We have followed the fate of radiolabeled anti-H-2K antibodies in target and control placentas that have absorbed these antibodies *in vivo*. The appearance of radiolabel associated with fragments smaller than intact IgG, within placental cells indicated the presence of IgG degradation products. Degradation products of the anti-H-2 antibody were seen in the cytoplasm of antigen-bearing placental cells one hour after the administration of antibody, whereas no degraded products were detectable in the control (non antigen-bearing) placental cells

until 6 hours after the administration of antibody. Obviously the placenta is capable of ingesting antibodies that are bound by their $F(ab)_2$ portion and then digesting these immunoglobulins into smaller fragments. S-200 chromatography performed on cytoplasmic extracts made from target placentas showed that the digestion of the ingested antibody continued after the first hour, giving rise to smaller fragments. The nature of these fragments is not known. Some degradation of antibodies was seen in the control placentas 6 hours after the injection of the antibody. Presumably, a proportion of the IgG that is bound by Fc receptors in the placenta, are ingested and broken down gradually. Williams *et al* (1971) studied the fate of rat IgG absorbed by rat visceral yolk sac cells and found that a portion of the radiolabeled IgG was rapidly degraded. Similarly, a proportion of the antibodies bound by Fc receptors in the placenta, may be degraded, while another portion of the antibodies may be transported across the cell. Wild (1979) suggests that Fc receptors on the human trophoblast cell surface bind the Fc portion of the IgG which then become associated with coated vesicles and transported across the cell in these vesicles. The IgG in these coated vesicles are segregated from other proteins that are destined for proteolysis within phagolysosomes. However, if an antibody binds to the cell by its Fab portions then these antibodies are presumably ingested into phagosomes which fuse with lysosomes after which the ingested proteins are enzymically digested (Wild, 1979). A similar mechanism may be envisaged for the murine placenta, where antibodies bound by their Fc end are protected and transported across, while antibodies bound by their $F(ab)_2$ end are unprotected and are degraded. The fate of anti-H-2 antibody in the placenta would therefore depend on its orientation on the cell surface; if the antibody is bound by its $F(ab)_2$ end, then the antibody would be wrongly oriented for transportation and would be degraded.

The ability of the placenta to degrade absorbed antibodies was compared with that of the liver of F_1 hybrid adults bearing the target H-2K antigens. Column chromatographic studies showed that the pattern and kinetics of antibody breakdown in these two tissues are different. These differences may be explained in two ways. The enzymic repertoires in the two tissues may be different, the placenta being a unique tissue, may be endowed with specialized proteases that may cleave proteins in a different manner. A more likely explanation is that the enzymes in both tissues are

identical, but may be present in different concentrations. Then the ability of the placenta to degrade antibodies faster, may be attributed to a higher concentration of enzymes involved in immunoglobulin degradation. This problem has not yet been resolved.

IgG breakdown products make their appearance in the serum of target pregnancies 3 hours before such breakdown products are seen in the serum of control pregnancies. This suggests that the degraded products seen in the serum of allogeneic pregnancies may originate from the allogeneic antigen-bearing conceptuses. It is highly likely that these breakdown products originate from target antigen-bearing placentas and not from the fetuses because it is clear that the placenta absorbs anti-fetal anntibodies from maternal circulation thus blocking the access of these antibodies into the fetus.

It is interesting that no evidence of antigen-antibody complexes was found in the serum. This does not entirely exclude the possibility of antibodies being released as complexes, because these antigen-antibody complexes may be sequestered soon after their release by phagocytes in the placenta, capable of binding antigen-antibody complexes.

We may conclude therefore that the primary fate of anti-H-2 antibodies absorbed by the placenta *in vivo*, is that of internalization and the subsequent digestion into fragments. A proportion of the breakdown products may then be released into maternal circulation and it has been suggested that another portion of the degraded protein may be reserved for supplying the fetus with essential amino acids (Wild, 1979).

V. CONCLUSIONS

Studies of allograft rejection have provided an insight into the dynamic interrelationships that exist between rejection reactions and facilitation mechanisms. A rejection reaction involves specific alloreactive cytotoxic T cells and specific cytotoxic antibodies, and a facilitation reaction usually consists of enhancing antibodies and suppressor cells. A dominance of the rejection reaction results in the facilitation reaction being surpassed and the allograft being rejected. Pregnancy, which has been referred to as nature's successful experiment in transplantation, is a situation where strong rejection reactions are lacking, but the experimental induction of strong anti-fetal reactions related to MHC antigens of the fetus does not result in rejection of the fetus. The placenta may thus be considered to be a naturally allografted tissue which through evolutionary processes has apparently discovered ways of surviving and being accepted immunologically by the mother, and it has also succeeded in developing ways of protecting and nurturing the fetus.

Firstly, the question of whether paternally-inherited MHC antigens are expressed at the maternal-fetal interface, has been conclusively answered in this thesis. Paternally-derived H-2K and H-2D (Class I MHC) antigens are present on spongiotrophoblast cells in intimate contact with maternal decidua and also bathed in maternal blood. I-A (Class II) antigens are not expressed, in detectable levels, in the placenta. A low level expression of Class I alloantigens coupled with the absence of Class II antigens may explain why intensive maternal cellular reactions against the fetus are absent. Suppressor cells or factors in the fetal environment may also contribute to this lack of strong rejection reactions in the mother. On the other hand, humoral antibody responses against fetal antigens, are often elicited during pregnancy. The results elaborated in this thesis, have demonstrated that the placenta plays a crucial role in absorbing and thus preventing these antibodies from entering the fetus. Thus the success of the fetal allograft may be attributed to the immunoabsorbent capabilities of the placenta.

The capacity of the placenta to absorb anti-paternal H-2 antibodies from the maternal circulation and to re-express its H-2 antigens has also been confirmed. Once maternal anti-fetal antibodies are absorbed from maternal circulation, the placenta ingests

and digests these antibodies and may release these digested products into the circulation. The net effect would be the elimination of potentially detrimental antibodies and the presentation once again, of antigens on the cell surface which can continue to perform its role as a sponge. This would obviously ensure the efficiency of the placental immunoabsorbent barrier.

The question of immunoregulation during pregnancy remain unresolved, because one can prime pregnant females against their allogeneic fetus and yet pregnancy is not compromised (Wegmann, 1981). Thus one again returns to the opening paragraph of the discussion i.e., pregnancy calls for some precisely coordinated physiological processes required for protecting the embryo from maternal immune rejection, and the unique immunological properties of the placenta appear to be primarily responsible for the lack of anticipated rejection reactions resulting from fetal–maternal histoincompatibility.

VI. REFERENCES

Abelev, G.I. 1975. Alpha-fetoprotein as a marker of embryo-specific differentiation in normal and tumor tissues. *Transplant. Rev.* **20**, 1.

Adinolfi, M. 1975. The human placenta as a filter for cells and plasma proteins. *in* Immunobiology of trophoblast. (Eds. Edwards, Howe and Johnson). p. 193. Cambridge University Press, Cambridge.

Alter, B.J., and Bach, F.H. 1979. Speculations on alternative pathways of T-lymphocyte response. *Scand. J. Immunol.* **10**, 87.

Amoroso, E.C. 1964. Histology of the placenta. *Brit. Med. Bull.* **17**, 81.

Amos, D.B., Gorer, P.A., Mikulska, B.M., Billingham, R.E., and Sparrow, E.M. 1954. An antibody response to skin homografts in mice. *Brit. J. Exp. Pathol.* **35**, 203.

Anderson, J.M. 1972. Nature's transplant: The transplantation immunology of viviparity. Butterworths. 1972.

Anderson, R.H., and Monroe, C.W. 1962. Experimental study of the behaviour of adult human skin homografts during pregnancy. *Amer. J. Obstet. Gynecol.* **84**, 1096.

Artzt, K., Bennett, D., and Jacob, F. 1974. Primary teratocarcinoma cells express a differentiation antigen specified by a gene at the T-locus in the mouse. *Proc. Natl. Acad. Sci.* **71**, 811.

Bach, F.H., Bach, M.L., and Sondel, P.M. 1976. Differential function of major histocompatibility antigens in the T-lymphocyte activation. *Nature* **259**, 273.

Baines, M.G., Speers, E.A., Pross, H., and Millar, K.G. 1976. Characteristics of the maternal lymphoid response of mice to paternal strain antigens induced by homologous pregnancy. *Immunol.* **31**, 363.

Barker, C.F., and Billingham, R.E. 1971. The lymphatic status of hamster cheek pouch tissue in relation to its properties as a graft and as a graft site. *J. Exp. Med.* **133**, 620.

Barnes, R.D., and Tuffrey, M. 1971. Maternal cells in the newborn. *in* Advances in the biosciences. **6**, 457. Pergamon Press, Vieweg.

Beer, A.E. 1980. Immunology of reproduction and embryonic development. *in* Immunology 80. Progress in Immunology. **IV**, 1137. (Eds. Fougereau and Dausset) Academic Press, London.

Beer, A.E., and Billingham, R.E. 1971. Immunobiology of mammalian reproduction. *Adv. Immunol.* **14**, 2.

Beer, A.E., and Billingham, R.E. 1973. Maternally acquired runt disease. Immune lymphocytes from the maternal blood can traverse the placenta and cause runt disease in the progeny. *Science*. **179**, 240.

Beer, A.E., and Billingham, R.E. 1974. Host responses to intrauterine tissue, cellular and fetal allografts. *J. Reprod. Fert.* **21**(suppl), 59.

Beer, A.E., and Billingham, R.E. 1976. The immunobiology of mammalian reproduction. Prentice-Hall Inc., New Jersey.

Beer, A.E., and Billingham, R.E. 1979. Maternal immunological recognition mechanisms during pregnancy. *in* Maternal recognition of pregnancy. Ciba Foundation series **64**, 293. Excerpta Medica, Amsterdam.

Beer, A.E., Billingham, R.E., and Scott, J.R. 1975. Immunogenetic aspects of implantation, placentation and fetal-placental growth rates. *Biol. Reprod.* **12**, 176.

Bell, S.C., and Billington, W.D. 1980. Major anti-paternal alloantibody induced by murine pregnancy is non-complement fixing IgG1. *Nature* **288**, 387.

Billingham, R.E. 1964. Transplantation immunity and the maternal-fetal relationship. *New Engl. J. Med.* **270**, 667.

Billingham, R.E. 1971. The passenger cell concept in transplantation immunology. *Cell. Immunol.* **2**, 1.

Billingham, R.E., and Silvers, W.K. 1964. Studies on homografts of foetal and skin and further observations on the anamolous properties of pouch skin grafts in hamsters. *Proc. R. Soc. Biol.* **61**, 168.

Billington, W.D. 1975. Organization, ultrastructure and histochemistry of the placenta: Immunological considerations. *in* Immunobiology of trophoblast. (Eds. Edwards, Howe and Johnson) Cambridge University Press, Cambridge.

Billington, W.D. 1976. The immunobiology of trophoblast. *in* Immunology of human reproduction. (Eds. Scott and Jones) p. 81. Academic Press, London.

Billington, W.D. 1979. The placenta and the tumour: Variations on an immunological enigma. *in* Placenta: A neglected experimental animal. (Eds. Beaconsfield and Villee). p. 267. Pergamon Press, London.

Billington, W.D., and Jenkinson, E.J. 1975. Antigen expression during early mouse development. *in* The early development of mammals. (Eds. Balls and Wild) p. 219. Cambridge University Press, Cambridge.

Billington, W.D., Jenkinson, E.J., Searle, R.F., and Sellens, M.H. 1977. Alloantigen expression during early embryogenesis and placental ontogeny in the mouse: immunoperoxidase and mixed hemadsorption studies. *Transpl. Proc.* **9**, 1371.

Billington, W.D., Kirby, D.R.S., Owen, J.J.T., Ritter, M.A., Burtonshaw, M.D., Evans, E.P., Ford, C.E., Gould, I.K., and McLaren, A. 1969. Placental barrier to maternal cells. *Nature* **224**, 704.

Breyere, E.J., and Barrett, M.K. 1960. Tolerance in primiparous female mice induced by strain-specific matings. *J. Natl. Cancer Inst.* **24**, 699.

Calarco, P.G., and Banka, C.L. 1979. Cell surface antigens of pre-implantation mouse embryos detected by an antiserum to an embryonal carcinoma cell line. *Biol. Reprod.* **20**, 699.

Caldwell, J.L., Stites, D.P., and Fudenberg, H.H. 1975. Human chorionic gonadotropin: Effects of crude and purified preparations on lymphocyte responses to phytohemagglutinin and allogeneic stimulation. *J. Immunol.* **115**, 1249.

Carr, M.C., Stites, D.P., and Fudenberg, H.H. 1973. Cellular immune aspects of the human fetal-maternal relationship. *Cell. Immunol.* **8**, 448.

Chaouat, G., Voisin, G.A., Escalier, D., and Robert, P. 1979. Facilitation reaction (enhancing antibodies and suppressor cells) from the mother to the paternal antigens of the foetus. *Clin. Exp. Immunol.* **35**, 13.

Chatterjee-Hasrouni, S., and Lala, P.K. 1979. Localization of H-2 antigens on mouse trophoblast cells. *J. Exp. Med.* **149**, 1238.

Chatterjee-Hasrouni, S., and Lala, P.K. 1981. MHC antigens on mouse trophoblast cells. *J. Immunol.* **127**, 2070.

Clark, D.A., and McDermott, M.R. 1978. Impairment of host versus graft reaction in pregnant mice. I. Suppression of cytotoxic T cell generation in lymph node draining the uterus. *J. Immunol.* **121**, 1389.

Clark, D.A., and McDermott, M.R. 1981. Active suppression of the host versus graft reaction in pregnancy. III. Developmental kinetics, properties and mechanisms of induction of suppressor cells during pregnancy. *J. Immunol.* **127**, 1267.

Clark, D.A., McDermott, M.R., and Szewcuk, M.R. 1980. Impairment of host versus graft reaction in pregnant mice. II. Selective suppression of cytotoxic T cell generation correlates with soluble suppressor activity and with successful allogeneic pregnancy. *Cell. Immunol.* **52**, 106.

Contractor, S.F., and Davies, H. 1973. Effect of human chorionic somatomammotropin and human chorionic gonadotropin on phytohemagglutinin induced lymphocyte transformation. *Nature.* **243**, 282.

Croy, B.A., Rossant, J., and Clark, D.A. 1982. Histological and immunological studies of postimplantation death of Mus caroli embryos in the Mus musculus uterus. *J. Reprod. Immunol.* **In press**.

Davies, J., and Glasser, S.R. 1968. Histological and fine structural observations on the

placenta of the rat. *Acta anat.* **69**, 542.

Delovitch, T.L., Press, J.L., and McDevitt, H.O. 1978. Expression of murine Ia antigens during embryonic development. *J. Immunol.* **120**, 818.

Desai, R.G., and Creger, W.P. 1963. Maternalfetal passage of leukocytes and platelets in man. *Blood* **21**, 665.

Doughty, R.W., and Gelsthorpe, K. 1976. Some parameters of lymphocyte antibody activity through pregnancy and eluates of placental material. *Tissue Antigens* **8**, 43.

Edidin, M. 1972. The appearance of cell surface antigens in the development of the mouse embryo: A study of cell surface differentiation. *in* Embryogenesis in mammals. Ciba Foundation Symposium 40. p. 177. American Elsevier Publication Co., New York.

Eddin, M. 1977. The mammalian embryo as a graft. *Clin. Obstet. Gynecol.* **20**, 669.

Edidin, M., Patthey, H.C., McGuire, E.J., and Sheffield, W.D. 1971. An antiserum to embryoid body tumour cells that reacts with normal mouse embryos. *in* Embryonic and fetal antigens in cancer. (Eds. Anderson and Coggin) Oak Ridge, Tennessee.

Enders, A.C. 1965. A comparative study of the fine structure of the trophoblast in several hemochorial placentas. *Amer. J. Anat.* **116**, 29.

Faulk, W.P., Jeannet, M., Creighton, W.D., and Carbonara, A. 1974. Immunological studies of the human placenta. Characterisation of immunoglobulins on the trophoblast basement membrane. *J. Clin. Invest.* **54**, 1011.

Faulk, W.P., Lovins, R.E., Yeager, C., and Temple, A. 1977. Antigens of human trophoblasts: Immunological and biochemical characterisation. *in* Immunological influence on human infertility. (Ed. Boettcher) p. 153. Academic Press, London.

Faulk. W.P., Temple, A., Lovins, R., Smith, N.C. 1978. Antigens on the human trophoblast: A working hypothesis for their role in normal and abnormal pregnancies. *Proc. Natl. Acad. Sci.* **25**, 1947.

Gill, T.J. III. 1977. Chimerism in humans. *Transplant. Proc.* **9**, 1423.

Gill, T.J. III. 1982. The embryo as antigen. *in* Reproductive Immunology. (Eds. Wegmann and Gill) Oxford University Press, New York.

Gill, T.J. III, and Repetti, C.F. 1979. Immunologic and genetic factors influencing reproduction. *Amer. J. Pathol.* **95**, 465.

Goodfellow, P.N., Barnstable, C.J., Bodmer, W.F., Snary, D., and Crumpton, M.J. 1976. Expression of HLA system antigens on the placenta. *Transplant.* **22**, 595.

Gooding, L.R., and Edidin, M. 1974. Cell surface antigens of mouse testicular teratomas. *J. Exp. Med.* **140**, 61.

Gorer, P.A. 1938. The antigenic basis of tumor transplantation. *J. Pathol. Bacteriol* **47**, 231.

Graff, R.J. 1978. Minor histocompatibility genes and their antigens. *Transplant. Proc.* **10**, 701.

Greenwood, F., Hunter, W., and Glover, J. 1963. The preparation of ^{131}I-human growth hormone of high specific radioactivity. *Biochem. J.* **89**, 114.

Gusdon, J.P. 1976. Maternal immune responses in pregnancy. *in* Immunology of human reproduction. (Eds. Scott and Jones) p. 103. Academic Press, London.

Hakansson, S., Heyner, S., Sundqvist, K.G., and Bergstrom, S. 1975. The presence of paternal H-2 antigens in hybrid mouse blastocysts during experimental delay of implantation and the disappearance of these antigens after the onset of implantation. *Internatl. J. Fertil.* **20**, 137.

Haldane, J.B.S. 1933. The genetics of cancer. *Nature* **132**, 265.

Harris, R.E., and London, R.E. 1976. The association of maternal lymphocytotoxic antibodies with obstetric complications. *Obstet. Gynecol.* **43**, 302.

Harrison, M.R. 1976a. Maternal immunocompetence. I. The g-v-h reativity of lymphocytes from pregnant rats and the distribution pattern of ^{51}Cr-labeled lymphocytes in pregnant mice. *Scand. J. Immunol.* **5**, 549.

Harrison, M.R. 1976b. Maternal immunocompetence. II. Proliferative response of maternal lymphocytes *in vitro* and inhibition by serum from pregnant rats. *Scand. J. Immunol.* **5**, 881.

Head, J.R., and Billingham, R.E. 1982. Transplantation Immunobiology revisited. *in* Reproductive Immunology. (Eds. Wegmann and Gill) Oxford University Press, New York. In press.

Hendricks, A.G., and Houston, M.L. 1970. Gestation and prenatal development. *in* Reproduction and breeding techniques for laboratory animals. (Ed. Hafez) Lea and Fabiger, Philadelphia.

Herzenberg, L.A., and Gonzales, B. 1962. Appearance of H-2 agglutinins in outcrossed female mice. *Proc. Natl. Acad. Sci.* **48**, 570.

Herzenberg, L.A., Bianchi, D.W., Schroder, J., Cann, H.M., and Iverson, G.M. 1979. Fetal cells in the blood of pregnant women. Detection and enrichment by fluorescence-activated cell sorting. *Proc. Natl. Acad. Sci.* **76**, 1453.

Heslop, R.W., Krohn, P.L., and Sparrow, E. 1954. The effect of pregnancy on the survival

of skin homografts in rabbits. *J. Endocrinol.* **10**, 325.

Heyner, S. 1973. Detection of H-2 antigens on the cells of the early mouse embryo. *Transplant.* **16**, 675.

Heyner, S. 1980. Antigens of trophoblast and early embryo. *in* Immunological aspects of fertility and fertility regulation. (Eds. Dhindsa and Schumacher) p. 183. Elsevier/North Holland, New York.

Heyner, S. 1982. Alloantigen expression on mouse oocytes and early embryos. *in* Reproductive Immunology. (Eds. Wegmann and Gill) Oxford University Press, New York. Heyner, S., Brinster, R.L., and Palm, J. 1969. Effect of alloantibodies on the preimplantation mouse embryo. *Nature* **222**, 783.

Heyner, S., and Hunziker, R.D. 1979. Differential expression of alloantigens of the MHC on unfertilized and fertilized eggs. *Dev. Genet.* **1**, 69.

Heyner, S., Hunziker, R.D., and Zink, G.L. 1980. Differential expression of minor histocompatibility antigens on the surface of the mouse oocyte and preimplantation developmental stages. *J. Reprod. Immunol.* **2**, 269.

Hulka, J.F., Brinton, V., Schaaf, J., and Baner, C. 1963. Appearance of antibodies to trophoblast during the postpartum period in normal human pregnancy. *Nature (Lond)* **198**, 501.

Irie, R.F., Giuiliano, A.E., and Morton, D.L. 1979. Oncofetal antigen: A tumor associated fetal antigen immunogenic in man. *J. Natl. Cancer. Inst.* **63**, 367.

Jacob, F. 1977. Mouse teratocarcinoma and embryonic antigens. *Immunol. Rev.* **33**, 3.

Jeannet, M., Werner, C., Ramirez, E., Vassalli, P., and Faulk, W.P. 1977. Anti-human Ia like and MLC blocking activity of human placental IgG. *Transplant. Proc.* **9**, 1417.

Jenkinson, E.J. 1981. Antigens and embryos. *Immunol. Today* **2**, 21.

Jenkinson, E.J., and Billington, W.D. 1974. Differential susceptibility of mouse trophoblast and embryonic tissue to immune cell lysis. *Transplant.* **18**, 286.

Jenkinson, E.J., and Owen, V. 1980. Ontogeny and distribution of major histocompatibility (MHC) antigens on murine placental trophoblast. *J. Reprod. Immunol.* **2**, 173.

Jenkinson, E.J., and Searle, R.F. 1979. Ia antigen expression on the developing mouse embryo and placenta. *J. Reprod. Immunol.* **1**, 3.

Jensen, C.O. 1903. cited by Klein, 1975.

Johnson, M., and Edidin, M. 1978. Lateral diffusion in plasma membranes of mouse egg is restricted after fertilization. *Nature* **272**, 448.

Kaliss, N., and Dagg, M.K. 1964. Immune response engendered in mice by multiparity. *Transplant.* **2**, 416.

Kirby, D.R.S., Billington, W.D., and James, D.A. 1966. Transplantation of eggs to the kidney and uterus of immunised mice. *Transplant.* **4**, 713.

Kitzmiller, J.L., and Rocklin, R.E. 1980. Lack of suppression of lymphocyte MIF production by estradiol, progesterone and human chorionic gonadotropin. *J. Reprod. Immunol.* **1**, 297.

Klein, J. 1975. Biology of the mouse Histocompatibility-2 complex. Springer-Verlag, New York.

Klein, J., Juretic, A., Baxevanis, C.N., and Nagy, Z.A. 1981. The traditional and a new version of the mouse H-2 complex. *Nature* **291**, 455.

Klein, J., and Park, J.M. 1974. Graft versus host reactions across different regions of the H-2 complex of the mouse. *J. Exp. Med.* **137**, 1213.

Klopper, A. 1980. The new placental proteins. A review. *Placenta* **1**, 77.

Krco, C.J., and Goldberg, E.H. 1977. Major histocompatibility antigens on pre-implantation mouse embryos. *Transplant. Rev.* **9**, 1367.

Lacy, P.E., Davie, J.M., and Finke, E.H. 1979. Prolongation of islet allograft survival following *in vitro* culture and a single injection of anti-lymphocyte serum. *Science* **203**, 312.

Lafferty, K.J. 1980. Immunogenicity of foreign tissues. *Transplant.* **29**, 179.

Lafferty, K.J., and Cunningham, A.J. 1975. A new analysis of allogeneic interactions. *Aust. J. Exp. Biol. Med. Sci.* **53**, 27.

Lafferty, K.J., and Woolnough, J. 1977. The origin and mechanism of the allograft reaction. *Immunol. Rev.* **35**, 231.

Lanman, J.T., Dinerstein, J., and Fikrig, S. 1962. Homograft immunity in pregnancy: Lack of harm to fetus from sensitization of mother. *Ann, N.Y. Acad. Sci.* **99**, 706.

Lawler, S.D., Klonda, P.T., and Bagshawe, K.D. 1974. Immunogenicity of molar pregnancies in the HLA system. *Amer. J. Obstet. Gynecol.* **120**, 857.

Little, C.C. 1924. The genetics of tissue transplantation. *J. Cancer Res.* **8**, 75.

Loeb, L. 1908. cited by Klein, 1975.

Loke, Y.W. 1978. Immunology and immunopathology of the human fetal-maternal

interactions. Elsevier/North-Holland Biomed Press, New York.

Loke, Y.W., Joysey, V.C., and Borland, R. 1971. HL-A antigens on human trophoblast cells. *Nature* **232**, 403.

Loveland, B.E., and McKenzie, I.F.C. 1982. Which T cells cause graft rejection? *Transplant.* **33**, 217.

Maroni, E.S., and deSousa, M.A.B. 1973. The lymphoid organs during pregnancy in the mouse: A comparison between a syngeneic and an allogeneic mating. *Clin. exp. immunol.* **13**, 107.

McLean, J.M., Mosley, J.G., and Gibbs, A.C.C. 1974. Changes in the thymus, spleen and lymph nodes during pregnancy and lactation in the rat. *J. Anat.* **118**, 223.

McIntyre, J.A., and Faulk, W.P. 1979. Role of the major histocompatibility complex in the immunobiology of transplantation antigens. *in* Placenta: A neglected experimental animal. (Eds. Beaconsfield and Villee) p. 294. Pergamon Press, New York.

Medawar, P.B. 1944. The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat.* **78**, 176.

Medawar, P.B. 1953. Some immunologic and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp. Soc. Exp. Biol.* **7**, 320.

Medawar, P.B., and Sparrow, E.M. 1956. The effect of adrenocortical hormones, adrenocorticotropic hormone and pregnancy on skin transplantation immunity in mice. *J. Endocrinol.* **14**, 240.

Mendenhall, H.W. 1976. The immunology of the fetal-maternal relationship. *in* Immunology of human rreproduction. (Eds. Scott and Jones) p. 61. Academic Press, London.

Mishell, R.I., Herzenberg, L.A., and Herzenberg, L.A. 1963. Leukocyte agglutinins in mice: Detection of H-2 and non H-2 isoagglutinins. *J. Immunol.* **90**, 628.

Mitchison, N.A. 1953. The effect on the offspring of maternal immunisation in mice. *J. Genet.* **51**, 406.

Moller, G. 1976. Biochemistry and biology of Ia antigens. *Transplant. Revs.* **30**, 117.

Montogomery, B., and Lala, P.K. 1981. HLA antigens on human trophoblast cells. *J. Reprod. Immunol.* (suppl), 57.

Morse, J.H., Stearns, G., Arden, J., and Canfeld, R.E. 1976. The effects of crude and purified human gonadotropin on *in vitro* stimulated human lymphocyte cultures. *Cell. Immunol.* **25**, 178.

Murgita, R.A. 1976. The immunosuppressive role of alpha-fetoprotein during pregnancy. *Scand. J. Immunol.* **5**, 1003.

Murgita, R.A., and Tomasi, T.B. Jr. 1975. Suppression of the immune response by alpha-fetoprotein. I. The effect of maternal alpha-fetoprotein on the primary and secondary antibody response. *J. Exp. Med.* **141**, 269.

Nissonoff, A., Wissler, F.C., Lipman, L.N., and Woernley, D.L. 1960. Separation of univalent fragments from the divalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys.* **89**, 230.

Oi, V.T., Jones, P.P., Goding, J.W., Herzenberg, L.A., and Herzenberg, L.A. 1978. Properties of monoclonal antibodies to mouse Ig allotypes and Ia antigens. *Curr. Top. Microbiol. Immunol.* **81**, 115.

O'Neill, H.C., and McKenzie, I.F.C. 1980. Quantitative variations in H-2 antigen expression. *Immunogenet.* **11**, 225.

Ozato, K., Mayer, N., and Sachs, D.H. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol.* **124**, 533.

Palm, J., Heyner, S., and Brinster, R.L. 1969. Differential immunofluorescence of fertilized mouse eggs with H-2 and non H-2 antibodies. *J. Exp. Med.* **133**, 282.

Patthey, H.L., and Edidin, M. 1973. Evidence for the time of appearance of H-2 antigens on mouse development. *Transplant.* **15**, 211.

Pavia, C.S., and Stites, D.P. 1979. Humoral and cellular regulation of alloimmunity in pregnancy. *J. Immunol.* **123**, 2194.

Pavia, C.S., Stites, D.P., and Fraser, R. 1981. Transplantation antigen expression on murine trophoblast: Detection by induction of specific alloimmunity. *Cell. Immunol.* **64**, 162.

Pellegrino, H.A., Pellegrino, A., and Kahan, B.D. 1970. Solubility of fetal HL-A antigens. *Transplant.* **10**, 425.

Raghupathy, R., Singh, B., Barrington Leigh, J., and Wegmann, T.G. 1981. Ontogeny and turnover kinetics of paternal H-2K determinants on the allogeneic murine placenta. *J. Immunol.* **127**, 2074.

Ray, P.K., and Seshadri, M. 1980. Influence of the parity status of female mice on the growth of a transplantable chemically inducible fibrosarcoma. *Ind. J. Exp. Biol.* **19**, 405.

Ray, P.K., Sengupta, J., Chakrabarti, P., and Saha, S. 1982. Sarcoma-180 tumors share common antigens with syngeneic embryos. Submitted for publication.

Revillard, J.P., Robert, M., DuPont, E., Betuel, H., Rivle, G., and Traegger, J. 1973. Inhibition

of mixed lymphocyte culture by alloantibodies in renal transplantation and in pregnancy. *Transplant. Proc.* **5**, 331.

Rocklin, R.E., Kitzmiller, J.L., Carpenter, C.B., Garovay, M.R., and David, J.R. 1976. Maternal–fetal relationship: Absence of an immunologic blocking factor from the serum of women with chronic abortions. *New Engl. J. Med.* **295**, 1209.

Rocklin, R.E., Kitzmiller, J.L., and Kaye, M.D. 1979. Immunobiology of the maternal–fetal relationship. *Amer. J. Pathol.* **95**, 465.

Rocklin, R.E., Zuckerman, J., Alper, E., and David, J.R. 1973. Effect of multiparity on human maternal hypersensitivity to fetal antigens. *Nature* **241**, 130.

Rossant, J., Mauro, V.M., and Croy, B.A. 1982. Importance of trophoblast genotype for survival of interspecific murine chimeras. *J. Embryol. exp. Morphol.* in press.

Rossant, J., and Papaioannou, V.E. 1977. The biology of embryogenesis. *in* concepts in mammalian embryogenesis. (Ed. Sherman). p 1. The MIT Press, London.

Rugh, R. 1968. The mouse: Its reproduction and development. Burgess Publishing Co., Minneapolis, MN. Schroder, J. 1975. Transplacental passage of blood cells. *J. Med. Genet.* **12**, 230.

Searle, R.F., Johnonson, M.H., Elson, J., and Clutterbuck–Jackson, S. 1974. Investigation of H–2 and non H–2 antigens on the mouse blastocyst. *Transplant.* **18**, 136.

Searle, R.F., Sellens, M.H., Elson, J., Jenkinson, E.J., and Billington, W.D. 1976. Detection of alloantigens during preimplantation development and early trophoblast differentiation in the mouse by immunoperoxidase labeling. *J. Exp. Med.* **143**, 328.

Sellens, M.H., Jenkinson, E.J., and Billington, W.D. 1978. MHC and non–MHC antigens on mouse ectoplacental cone and trophoblast cells. *Transplant.* **25**, 173.

Sheppard, H.W. Jr., Sell, S., Trefts, P., and Balu, R. 1977. Effects of alpha–fetoprotein on murine immune response. I. Studies on mice. *J. Immunol.* **119**, 1977.

Siiteri, P.K., Febres, F., Clemens, L.E., Chang, R.J., Gondos, B., and Stites, D.P. 1977. Progesterone and maintenance of pregnancy: is progesterone nature's immunosuppressant? *Ann. N.Y. Acad. Sci.* **286**, 384.

Simmons, R.L., and Russell, P.S. 1967. Immunologic interactions between mother and fetus. *Adv. Obstet. Gynec.* **1**, 38.

Slapsys, R.M., and Clark, D.A. 1982. Active local suppression of host versus graft reactions in pregnant mice IV. Local suppressor cells in decidua and uterine blood. *J. Reprod. Immunol.* In Press. Federation of Biological Societies meeting, Edmonton.

Smith, J.A., Burton, R.C., Barg, M., and Mitchell, G.F. 1978. Maternal alloimmunization in pregnancy. *In vitro* studies of T cell-dependent immunity to paternal alloantigens.

Solter, D., and Knowles, B.B. 1978. Monoclonal antibodies defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc. Natl. Acad. Sci.* **75**, 5565.

Solter, D., and Knowles, B.B. 1979. Developmental stage-specific antigens during mouse embryogenesis. *Curr. Top. Dev. Biol.* **13**, 139.

Stites, D.P., Pavia, C.S., Clemens, L.E., Kuhn, R.W., and Siiteri, P.K. 1979. Immunologic regulation in pregnancy. *Arth. Rheum.* **22**, 1300.

Strominger, J.L. 1980. Structure and products of the major histocompatibility complex in man and mouse. *in* Immunology 80. Progress in Immunology IV. (Eds. Fougereau and Dausset) p 542.

Sunderland, C.A., Redman, C.W.G., and Stirrat, G.M. 1981. HLA-A, B, C antigens are expressed on nonvillous trophoblast of the early human placenta. *J. Immunol.* **127**, 2614.

Swinburne, L.M. 1970. Leucocyte antigens and placental sponge. *Lancet* **2**, 592.

Takano, K., and Miller, J.R. 1972. ABO incompatibility as a cause of spontaneous abortions: Evidence from abortions. *J. Med. Genet.* **9**, 144.

Terasaki, P.I., Mickey, M.R., Yamazaki, J.N., and Vredevoe, D. 1970. Maternal-fetal incompatibility. I. Incidence of HL-A antibodies and possible association with congenital anomalies. *Transplant.* **9**, 538.

Terry, W.D., Henkart, P.A., Coligan, J.E., and Todd, C.W. 1975. Carcinoembryonic antigen. *Transplant. Rev.* **20**, 100.

Thorsby, E., Albrechtsen, D., Bergholtz, B.O., Hirschberg, H., and Solheim, B.G. 1978. Identification and significance of products of the HLA-D region. *Transplant. Proc.* **10**, 313.

Tongio, M.M., Mayer, S., and Lebec, A. 1975. Transfer of HLA antibodies from the mother to child. *Transplant.* **20**, 163.

Tuffrey, M., Bishun, N.P., and Barnes, R.D. 1969. Porosity of the placenta to maternal cells in normally derived mice. *Nature* **224**, 701.

Tyzzer, E.E. 1909. cited by Klein, 1975.

Voisin, G.A., and Chaoaut, G. 1974. Demonstration, nature and properties of antibodies fixed on placenta and directed against paternal antigens. *J. Reprod. Immunol.* **21** (suppl), 119.

Webb, C.G., Gall, W.E., Edelman, G. 1977. Synthesis and distribution of H-2 antigens in pre-implantation mouse embryos. *J. Exp. Med.* **146**, 923.

Wegmann, T.G. 1981. The presence of Class I mHC antigens at the maternal-fetal interface and hypothesis concerning the survival of the murine fetal allograft. *J. Reprod. Immunol.* **3**, 267.

Wegmann, T.G., Barrington Leigh, J., Carlson, G.A., Mosmann, T.R., Raghupathy, R., and Singh, B. 1980. Quantitation of the mouse placenta to absorb monoclonal antifetal H-2K antibodies. *J. Reprod. Immunol.* **2**, 53.

Wegmann, T.G., Mosmann, T.R., Carlson, G.A., Olijnyk, O., Singh, B. 1979a. The ability of the murine placenta to absorb monoclonal anti-fetal H-2K antibodies from the maternal circulation. *J. Immunol.* **123**, 1020.

Wegmann, T.G., Singh, B., Carlson, G.A. 1979b. Allogeneic placenta is a paternal strain antigen immunoabsorbent. *J. Immunol.* **122**, 270.

Wegmann, T.G., Waters, C.A., Drell, D.W., and Carlson, G.A. 1979c. Pregnant mice are not primed but can be primed to fetal aloantigens. *Proc. Natl. Acad. Sci.* **76**, 2410.

Weintraub, B.D., and Rosen, S.W. 1971. Ectopic production of human chorionic somatomammotropin by non-trophoblastic cancers. *J. Clin. Endocrinol. Metabol.* **32**, 94.

Wild, A.E. 1979. Placental antibody transport and immune protection- their cellular mechanisms. *in* Placenta: A neglected experimental animal. p 306. (Eds. Beaconsfield and Villee) p 306. Pergamon Press, London.

Wiley, L.M. 1979. Early embryonic cell surface antigens as developmental probes. *Curr. Top. Dev. Biol.* **13**, 167.

Wiley, L.M., and Calarco, P.G. 1975. The effects of anti-embryo sera and their localization on the cell surface during mouse preimplantation development. *Dev. Biol.* **47**, 407.

Williams, K.E., Lloyd, J.B., Davies, M., and Beck, F. 1971. Digestion of an exogenous protein by rat yolk-sac cultured *in vitro*. *Biochem. J.* **125**, 303.

Winn, H.J., Baldamus, C.A., Jooste, S.W., and Russell, P.S. 1973. Acute destruction by humoral antibody of rat skin grafted to mice. The role of complement and polymorphonuclear granulocytes. *J. Exp. Med.* **137**, 893.

Wood, G.W. 1980. Immunohistological identification of macrophages in the murine placenta, yolk-sac membranes and pregnant uterii. *Placenta* **1**, 1309.

Woodrow, J.C. 1965. Rh immunization and its prevention. *Ser. Haematol.* **3**, 2.

Youtananukorn, V., and Matangkasombut, P. 1972. Human maternal cell-mediated immune reaction to placental antigens. *Clin. exp. immunol.* 11, 549.